PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07C 311/06 | A1

(11) International Publication Number: WO 95/15308

(43) International Publication Date: 8 June 1995 (08.06.95)

(21) International Application Number: PCT/EP94/03750

(22) International Filing Date: 9 November 1994 (09.11.94)

(30) Priority Data:
9324931.6 4 December 1993 (04.12.93) GB

(71) Applicant (*for GB only*): PFIZER LIMITED [GB/GB]; Ramsgate Road, Sandwich, Kent CT13 9NJ (GB).

(71) Applicant (*for all designated States except GB JP US*): PFIZER RESEARCH AND DEVELOPMENT COMPANY, N.V./S.A. [BE/IE]; Alexandra House, Earlsfort Centre, Earlsfort Terrace, Dublin (IE).

(71) Applicant (*for JP only*): PFIZER INC. [US/US]; 235 East 42nd Street, New York, NY 10017 (US).

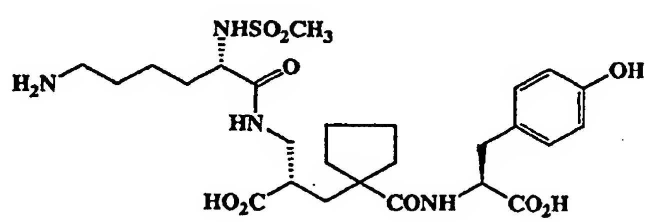
(72) Inventors; and
(75) Inventors/Applicants (*for US only*): DUNN, Peter, James [GB/GB]; Pfizer Central Research, Ramsgate Road, Sandwich, Kent CT13 9NJ (GB). HUGHES, Michael, Leslie [GB/GB]; Pfizer Central Research, Ramsgate Road, Sandwich, Kent CT13 9NJ (GB).

(74) Agents: MOORE, James, William et al.; Pfizer Limited, European Patent Dept., Ramsgate Road, Sandwich, Kent CT13 9NJ (GB).

(81) Designated States: AU, BR, CA, CN, CZ, FI, HU, JP, KR, NO, NZ, PL, RU, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: CRYSTALLINE, POLYMORPHIC FORM OF (S,S,S)-N-(1-[2-CARBOXY-3-(N2-MESYLLYSYLAMINO)PROPYL]-1-CYCLOPENTYLCARBONYL)TYROSINE



(I)

(57) Abstract

The present invention relates to a crystalline, α-polymorphic form of a compound of formula (I) and to processes for the preparation of, to intermediates used in the preparation of, to compositions containing and to uses of, the α-polymorphic form.

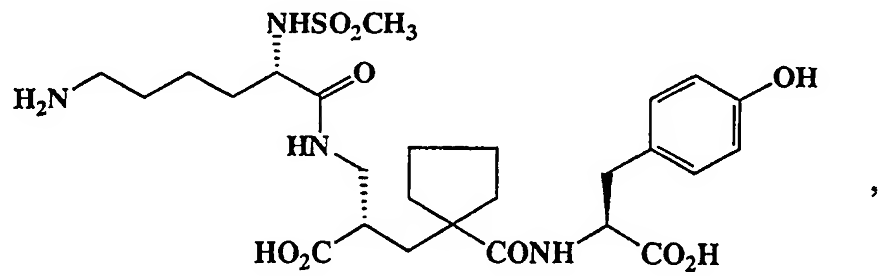
FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgystan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo			SE	Sweden
CH	Switzerland	KR	Republic of Korea	SI	Slovenia
CI	Côte d'Ivoire	KZ	Kazakhstan	SK	Slovakia
CM	Cameroon	LI	Liechtenstein	SN	Senegal
CN	China	LK	Sri Lanka	TD	Chad
CS	Czechoslovakia	LU	Luxembourg	TG	Togo
CZ	Czech Republic	LV	Latvia	TJ	Tajikistan
DE	Germany	MC	Monaco	TT	Trinidad and Tobago
DK	Denmark	MD	Republic of Moldova	UA	Ukraine
ES	Spain	MG	Madagascar	US	United States of America
FI	Finland	ML	Mali	UZ	Uzbekistan
FR	France	MN	Mongolia	VN	Viet Nam
GA	Gabon				

CRYSTALLINE, POLYMORPHIC FORM OF (S,S,S)-N-(1-[2-CARBOXY-3-(N2-MESYLLYSYLAMINO) PROPYL]-1-CYCLOPENTYLCARBONYL)TYROSINE

The present invention relates to a crystalline, polymorphic form of (S,S,S)-N-(1-[2-carboxy-3-(N^2-mesyllysylamino)propyl]-1-cyclopentylcarbonyl)tyrosine which has the formula:-



(I)

hereafter referred to as the "α-form" of a compound of the formula (I).

More particularly, the invention relates to the α-form of a compound of the formula (I) and to processes for the preparation of, to intermediates used in the preparation of, to compositions containing and to uses of, the α-form.

An amorphous form (hereafter referred to as the "ß-form") of a compound of the formula (I) has been disclosed in European Patent Publication No. EP-A-0358398 as Example 181. The compound is a potent inhibitor of the zinc dependent neutral endopeptidase E.C.3.4.24.11 and is therefore able to potentiate the biological effects of atrial natriuretic factor. It is therefore a natriuretic, antihypertensive and diuretic agent that is useful for the treatment of various cardiovascular disorders. The compound is also a potent inhibitor of angiotensin converting enzyme, a further enzyme that is involved in the control of blood pressure. The compound therefore has a dual pharmacological action through being capable of inhibiting two key enzymes involved in the control of blood pressure. It is therefore likely to

be useful in the treatment of various forms of hypertension and associated cardiovascular disorders such as congestive heart failure and glaucoma.

The ß-form can be obtained by methods such as freeze drying of a solution of the compound of the formula (I), by rapid evaporation of the solvent from such a solution or by precipitation from such a solution by addition of a poor solvent. The ß-form does not melt sharply but normally "softens" at about 160°C.

The ß-form has, however, been found to have certain properties which do not make it particularly suitable for pharmaceutical formulation. In particular it is hygroscopic in nature, it has a low bulk density and poor flow properties. Processing experiments carried out using the ß-form have revealed problems in manufacturing tablets from compositions containing this form.

The problem addressed by the present invention is the provision of a form of the compound of the formula (I) which can be efficiently processed to provide a stable and effective formulation of the drug.

This problem has been solved by the surprising finding that an α-form of a compound of the formula (I) can be prepared which is non-hygroscopic, crystalline and, when compared to the ß-form, which has a higher bulk density and better flow properties. The α-form is particularly suitable for use in pharmaceutical formulation of the drug.

The present invention therefore provides a crystalline, polymorphic α-form of a compound of the formula (I) which has an infra-red spectrum as a mull in nujol which shows absorption bands at ν = 3407, 3386, 3223, 3153, 1699, 1652, 1626, 1594, 1516, 1457 (nujol), 1377 (nujol), 1344, 1334, 1317, 1267, 1241, 1228, 1210, 1164, 1151, 1137, 1118, 1109, 1093, 1074, 1045, 1019, 1003, 981, 965, 911, 897, 862, 818, 800, 778, 762, 721 and 655 cm^{-1}.

The α-form is further characterised by its powder X-ray diffraction pattern obtained using copper radiation filtered with a graphite monochromator (λ = 0.15405nm) which shows main peaks at 7.5, 8.9, 9.9, 11.6, 15.6, 17.2, 17.5, 18.0, 20.2, 22.1 and 23.3 degrees 2θ.

The α-form is yet further characterised by differential scanning calorimetry in which it shows a sharp endotherm in the range 248-259°C and decomposes at above 260°C when subjected to a scanning rate of 20°C per minute.

The α-form typically melts sharply in the range 242-252°C, although lower melting point ranges have been recorded.

Other forms (hereafter referred to as the "γ-" and "δ-forms") of a compound of the formula (I) have also been obtained which also form part of the present invention since they can be used as intermediates in the preparation of the α-form.

The invention thus further provides a polymorphic γ-form of a compound of the formula (I) which has an infra-red spectrum as a mull in nujol which shows absorption bands at ν = 3377, 3240, 1665, 1639, 1594, 1527, 1518, 1494, 1457 (nujol), 1443, 1377 (nujol), 1344, 1321, 1304, 1254, 1195, 1178, 1162, 1143, 1111, 1098, 1046, 1031, 1012, 972, 962, 945, 932, 907, 879, 849, 815, 806, 780, 753, 729 and 658 cm^{-1}.

The γ-form is further characterised by its powder X-ray diffraction pattern obtained using copper radiation filtered with a graphite monochromator (λ = 0.15405nm) which shows main peaks at 9.0, 9.6, 10.6, 11.6, 12.7, 13.3, 14.6, 16.2, 17.9, 18.8, 20.2 and 21.8 degrees 2θ.

The γ-form is yet further characterised by differential scanning calorimetry in which it shows a sharp endotherm in the range 176-186°C, an exotherm at about 207°C and a weak endotherm at about 213°C and decomposes at above 250°C when subjected to a scanning rate of 20°C per minute.

The γ-form typically melts sharply in the range 170-185°C.

The invention thus also provides a hydrated δ-form of a compound of the formula (I) which has a water content of from 1 to 7%, preferably of from 2 to 4%, by weight, as determined by Karl Fischer analysis, and which has an infra-red spectrum as a mull in nujol which shows absorption bands at ν = 3667, 3425, 3380, 3287, 3137, 3098, 1709, 1673, 1637, 1619, 1596, 1568, 1556, 1516, 1458 (nujol), 1448, 1419, 1390, 1378 (nujol), 1356, 1338, 1300, 1270, 1249, 1229, 1198, 1174, 1141, 1108, 1091, 1075, 1064, 1045, 1033, 1019, 1001, 985, 962, 941, 909, 889, 877, 841, 822, 807, 763, 744, 732, 721 and 655 cm^{-1}.

The δ-form is further characterised by its powder X-ray diffraction pattern obtained using copper radiation filtered with a graphite monochromator (λ = 0.15405nm) which shows main peaks at 10.5, 10.8, 12.3, 14.5, 17.2, 17.6, 17.9, 18.9, 20.4, 21.5, 22.4, 23.0, 23.1, 24.7, 27.1, 27.8 and 28.9 degrees 2θ.

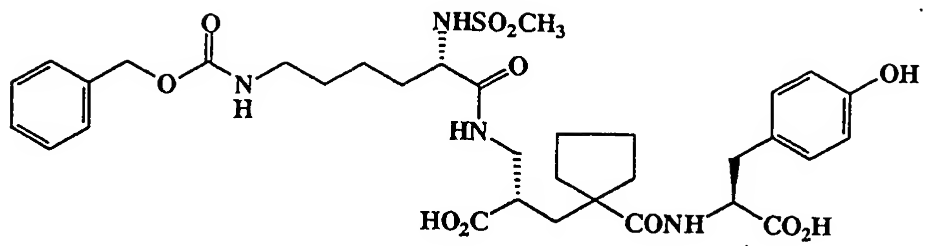
The δ-form is yet further characterised by differential scanning calorimetry in which it shows sharp endotherms at about 162°C and at about 166-168°C and decomposes at above 200°C when subjected to a scanning rate of 20°C per minute.

The δ-form typically melts sharply in the range 165-175°C.

Although the γ- and δ-forms of a compound of the formula (I) display the same pharmacological activities as the α- and β-forms, they are not as suitable as the α-form for the purpose of pharmaceutical formulation.

The α-form of a compound of the formula (I) can be prepared by the following methods:-

1) The α-form can be prepared by catalytic hydrogenation of an aqueous solution of a sodium, potassium, ammonium or (C_1-C_4 alkyl)ammonium salt of a compound of the formula:-



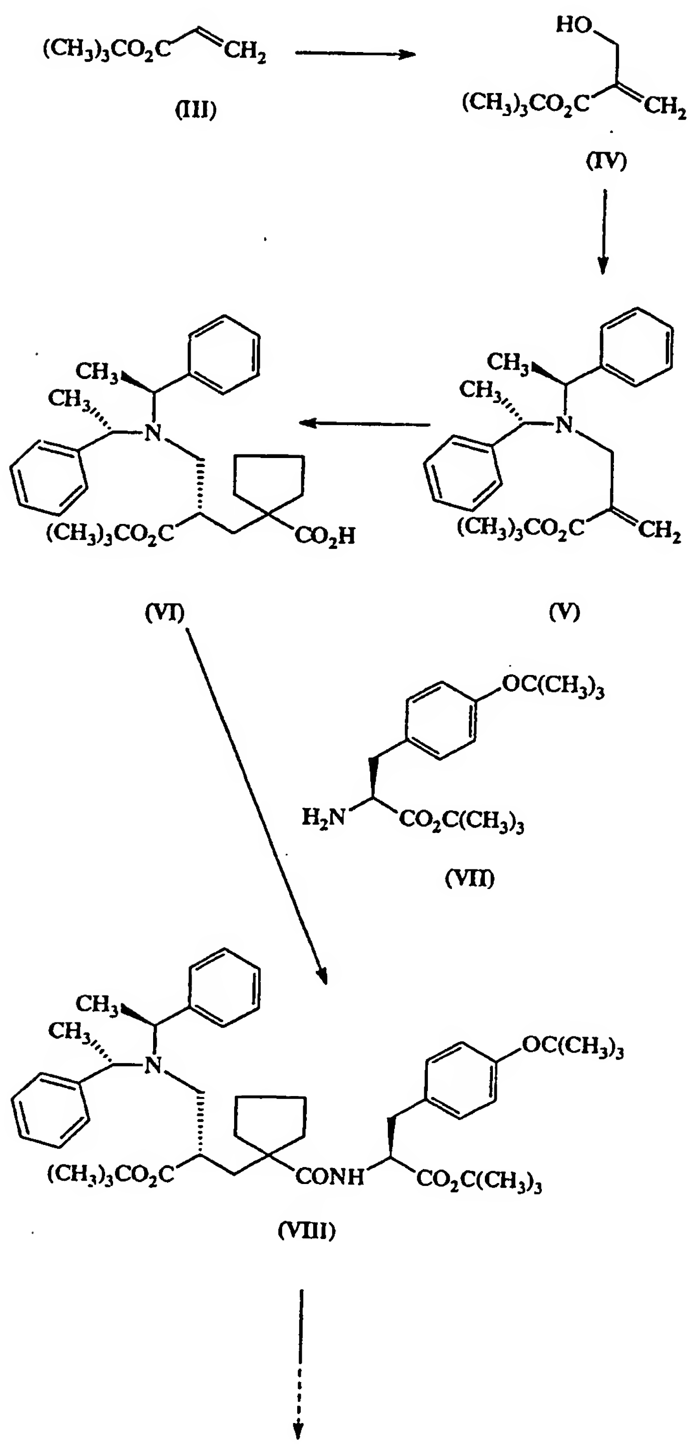
(II)

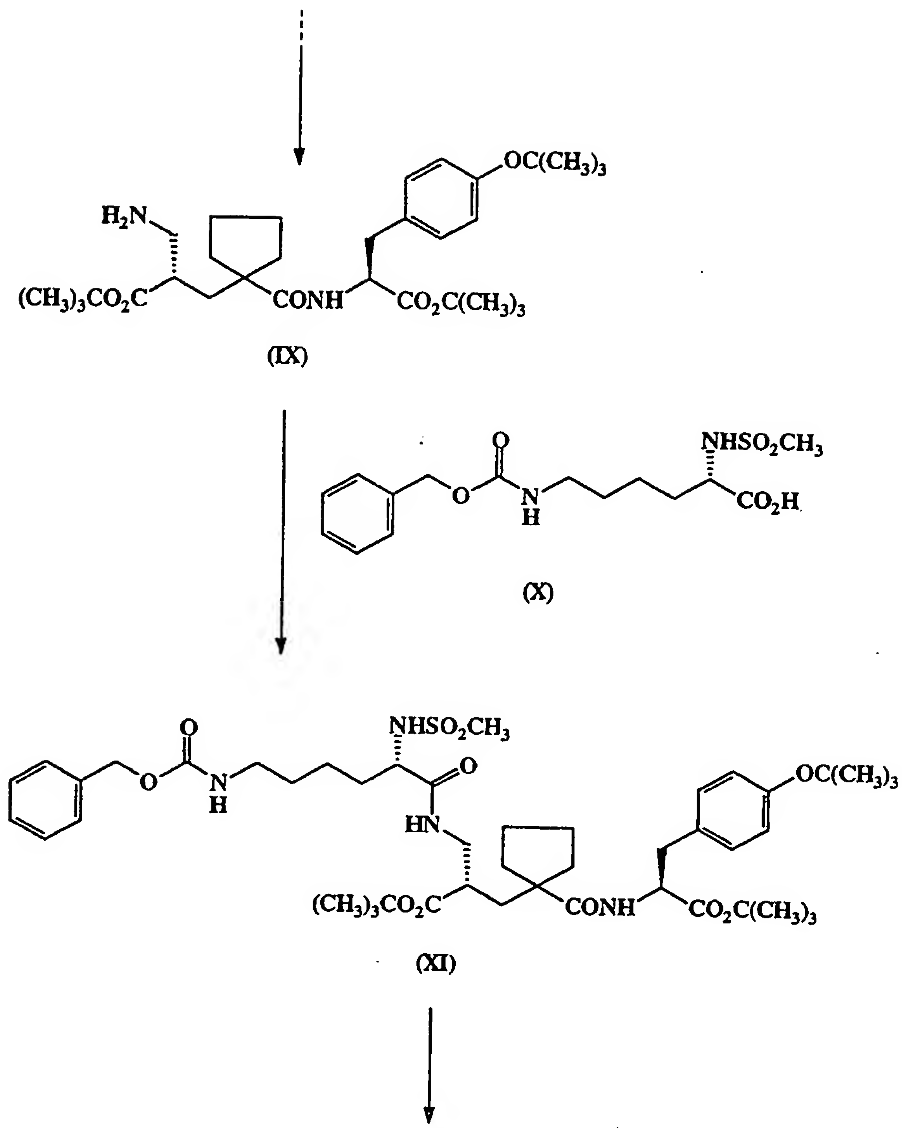
using a suitable catalyst for the removal of the benzyloxycarbonyl protecting group, e.g. palladium-on-carbon, followed by acidification of the base salt of the compound of the formula (I) obtained to from pH 3 to 5, preferably to about pH4, and preferably at from 35 to 45°C, to provide the α-form. Preferably a disodium salt of a compound of the formula (II) is used. Further suitable catalysts for the removal of the benzyloxycarbonyl protecting group are well known to the skilled person, e.g. see T.W. Greene and P.G. Wuts, "Protective Groups in Organic Synthesis", Second Edition, 1991, the teaching of which is incorporated herein by reference.

In a typical procedure, a solution of a compound of the formula (II) in a suitable organic solvent, e.g. ethyl acetate, is shaken with an aqueous solution of sodium hydroxide to generate a disodium salt thereof. The aqueous solution containing the sodium salt is then separated and hydrogenated in the presence of a 5% palladium-on-carbon catalyst at about 414kPa (60 psi) and room temperature to remove the benzyloxycarbonyl protecting group. The catalyst is then removed by filtration and the filtrate adjusted to about pH 4 using a suitable acid, e.g. aqueous hydrochloric acid. The α-form is precipitated from solution and can be collected by filtration.

A compound of the formula (II) can be prepared by the route set out in Scheme 1.

Scheme 1





A compound of the formula (II)

In a typical procedure, t-butyl acrylate (III) is reacted with paraformaldehyde in the presence of 3-quinuclidinol to provide t-butyl hydroxymethylacrylate (IV). This is first treated with thionyl chloride in the presence of triethylamine and pyridine to provide the corresponding chloromethylacrylate, which is then reacted with (S,S)-α-α'-dimethyldibenzylamine to provide an acrylate of the formula (V). This is converted to a compound of the formula (IX) by the method set out in Tet. Lett., 1993, <u>34</u>(8), 1323-6. A compound of the formula (IX) is then condensed with a lysine derivative of the formula (X) by a similar procedure to that described in EP-A-0358398 for the preparation of a compound of the formula (XI). A compound of the formula (XI) is then converted to a compound of the formula (II) using a solution of trifluoroacetic acid and anisole in dichloromethane.

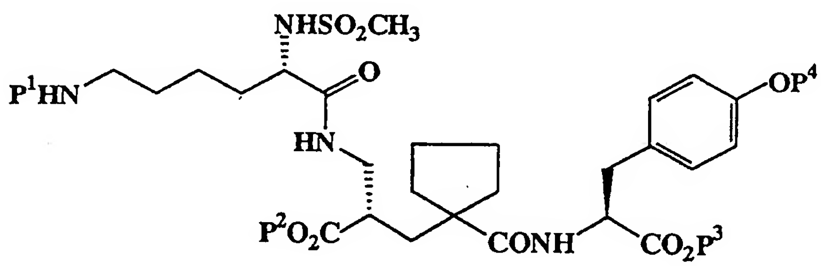
2) The α-form can be prepared from the δ-form by stirring a solution of the δ-form in water or in an aqueous solution of a suitable organic solvent, e.g. a C_1-C_4 alkanol such as methanol or isopropanol, or a C_3-C_6 alkanone such as acetone.
In a typical procedure the δ-form is dissolved in a 1:5 water/methanol or a 1:10 water/acetone mixture and the solution stirred for several days at room temperature. The α-form precipitates from the solution and can be collected by filtration.

3) The α-form can be prepared from the γ-form by stirring a solution of the γ-form in water or in an aqueous solution of a suitable organic solvent, e.g. a C_1-C_4 alkanol such as methanol or isopropanol, or a C_3-C_6 alkanone such as acetone.

In a typical procedure the γ-form is dissolved in a 1:1 water/methanol mixture and the solution stirred for about 17 hours at room temperature. The α-form precipitates from the solution and can be collected by filtration.

4) The α-form can be prepared from the ß-form by a similar procedure to that set out in Method (3) above.

5) The α-form can be prepared by deprotection, preferably under acidic conditions, of a compound of the formula:



(XII)

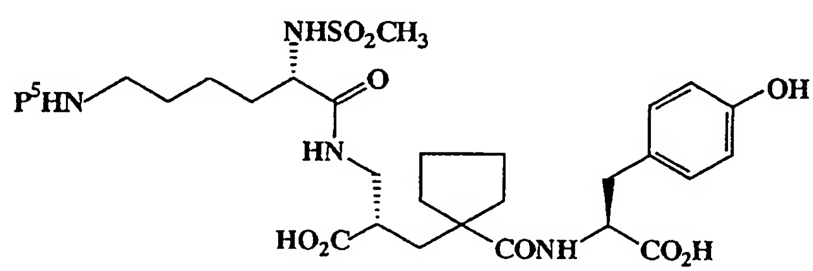
wherein P^1, P^2, P^3 and P^4 are all suitable protecting groups that are capable of removal, preferably under acidic conditions, to provide, following adjustment of the pH to from 3 to 5, preferably about 4, in the work-up, the α-form.

Suitable protecting groups for this purpose together with conditions for their removal will be well known to the skilled person, e.g. see T.W. Greene, and P.G. Wuts, "Protective Groups in Organic Synthesis", Second Edition, Wiley-Interscience. P^1 is preferably formyl or benzyloxycarbonyl. P^2, P^3 and P^4 are preferably each t-butyl.

In a typical procedure where P^1 is formyl or benzyloxycarbonyl and P^2, P^3 and P^4 are each t-butyl, a solution of a compound of the formula (XII) in a suitable solvent, e.g. 1,4-dioxane or ethyl acetate, is treated with a suitable acid, e.g. hydrogen chloride, to remove to the protecting groups and adjustment of the pH to about 4 in the work-up provided the α-form.

The intermediates of the formula (XII) may be prepared by conventional techniques. The compound of the formula (XII) where P^1 is benzyloxycarbonyl and P^2, P^3 and P^4 are each t-butyl corresponds to the compound of the formula (XI) in Scheme 1, the synthesis of which is further described in Method (1). The compound of the formula (XII) where P^1 is formyl and P^2, P^3 and P^4 are each t-butyl may be prepared by first removing the benzyloxycarbonyl group from the compound of the formula (XI) by hydrogenolysis using a suitable catalyst, e.g. palladium-on-carbon, followed by formylation of the amine obtained, e.g. using formic acetic anhydride.

6) The α-form can be prepared by deprotection, preferably under acidic conditions, of a compound of the formula:-



(XIII)

wherein P^5 is a suitable protecting group that is capable of removal, preferably under acidic conditions, to provide, following adjustment of the pH to from 3 to 5, preferably about 4, in the work-up, the α-form. Suitable protecting groups for this purpose together with conditions for their removal will be well known to the skilled person, e.g. see T.W. Greene and P.G. Wuts, "Protective Groups in Organic Synthesis", Second Edition, Wiley-Interscience. P^5 is preferably formyl and further examples of P^5 are benzyloxycarbonyl and tert-butyloxycarbonyl.

In a typical procedure where P^5 is formyl, a solution of a compound of the formula (XIII) in a suitable solvent, e.g. 1,4-dioxane, is treated with an aqueous solution of a suitable acid, e.g. hydrochloric acid, to remove the protecting group and adjustment of the pH to about 4 in work-up provided the α-form.

The intermediates of the formula (XIII) may be prepared by conventional techniques, such as by selective deprotection of a compound of the formula (XII) to remove the P^2, P^3 and P^4 protecting groups alone. For example, where P^1 is formyl and P^2, P^3 and P^4 are each t-butyl, the t-butyl protecting groups may be selectively removed by treatment of a compound of the formula (XII) with trifluoroacetic acid in a suitable solvent, e.g. dichloromethane.

The β-, γ- and δ-forms that are used as intermediates in preparing the α-form can be prepared as follows:-

(i) The ß-form can be prepared by catalytic hydrogenation of a solution of a compound of the formula (II) in a suitable solvent and in the presence of a suitable catalyst for the removal of the protecting group, e.g. palladium-on-carbon.
In a typical procedure, a solution of a compound of the formula (II) in aqueous ethanol is hydrogenated at about 414kPa (60 psi) and room temperature in the presence of a palladium-on-carbon catalyst. The catalyst is then removed by filtration and the filtrate is either concentrated under reduced pressure to provide a foam that is stirred with a C_3-C_6 alkanone, e.g. acetone, or freeze dried, to provide the ß-form that can be collected by filtration.
This preparation has also, if the C_3-C_6 alkanone treatment is used, occasionally provided the α-form.

(ii) The δ-form can be prepared by catalytic hydrogenation of a solution of a compound of the formula (II) in a mixture of a suitable water immiscible organic solvent, e.g. ethyl acetate, and water and in the presence of a suitable catalyst for the removal of the protecting group, e.g. palladium-on-carbon, followed by removal of the catalyst, separation of the aqueous layer and precipitation of the product from the aqueous layer using a C_1-C_4 alkanol, e.g. methanol.
In a typical procedure, water is added to a solution of a compound of the formula (II) in ethyl acetate and the mixture is hydrogenated at about 414kPa (60 psi) and room temperature in the presence of a palladium-on-carbon catalyst. The catalyst is then removed by filtration, the aqueous phase separated from the filtrate, concentrated under reduced pressure to a low volume and poured into methanol. The δ-form slowly precipitates from the solution and can be collected by filtration.

This preparation has also occasionally provided the α-form.

(iii) The ß-form can be prepared by first freezing an aqueous solution of the δ-form and then freeze drying the resulting solid mass.

(iv) The γ-form can be prepared by stirring the δ-form with n-propanol or acetonitrile.
In a typical procedure the mixture is stirred for about 24 hours at room temperature and the γ-form is collected by filtration.

(v) The γ-form can be prepared by stirring a slurry of the ß-form in acetonitrile or n-propanol, typically for about 5 days at room temperature. The γ-form is collected by filtration.

(vi) The γ-form can be prepared by treating an aqueous solution of the δ-form with a C_3-C_6 alkanone, e.g. acetone.
In a typical procedure an aqueous solution of the δ-form is poured into a vigorously stirred volumetric excess of acetone at room temperature. The γ-form precipitates from solution and can be collected by filtration.
This preparation has also occasionally provided the α-form.

(vii) The ß-form can be prepared by freeze drying a concentrated, aqueous solution of the α-form.
In a typical procedure, a concentrated solution of the α-form in hot water is prepared, the solution filtered to remove any insoluble material, then cooled, frozen and finally freeze dried to provide the ß-form.

As previously mentioned, the α-form is a potent inhibitor of the neutral endopeptidase (E.C.3.4.24.11). This enzyme is involved in the breakdown of a number of peptide hormones and peptide autocoid substances including, in particular, the breakdown of atrial natriuretic factor (ANF). Thus the α-form, by preventing the degradation of ANF by neutral endopeptidase E.C.3.4.24.11, can potentiate the biological effects of ANF and is therefore a diuretic, natriuretic and antihypertensive agent of utility in the treatment of a number of disorders including hypertension, heart failure, angina, renal insufficiency, chronic renal failure, premenstrual syndrome, cyclical oedema, Menieres disease, hyperaldosteroneism (primary and secondary) and hypercalciuria. In addition, because of its ability to potentiate the effects of ANF, the α-form is useful in the treatment of glaucoma. Further, as a result of its ability to inhibit the neutral endopeptidase E.C.3.4.24.11, the α-form may be useful in treating asthma, inflammation, pain, epilepsy, affective disorders, dementia, geriatric confusion, obesity, gastrointestinal disorders (especially diarrhoea and irritable bowel syndrome) and hyperreninaemia and in the modulation of gastric acid secretion.

The activity against neutral endopeptidase E.C.3.4.24.11 can be assessed using a procedure based on the assay described by Barclay, P.L., et al, Biochem. Biophys. Res. Comm., 1989, 164, 58-65. The method involves determining the concentration of compound required to reduce by 50% the rate of release of radiolabelled hippuric acid from hippuryl-L-phenylalanyl-L-arginine by a neutral endopeptidase preparation from rat kidney.

As previously mentioned, the α-form is also an inhibitor of angiotensin converting enzyme (ACE). As such it is useful in treating a variety of conditions for which ACE inhibitors are known to be useful including hypotension, congestive heart failure, limitation of ischaemic damage to the myocardium, protection of the kidney against hyperfiltration damage, prevention or reversal of left ventricular hypertrophy, memory enhancement, control of cognitive function, dementia and preventing reocclusion following coronary angioplasty or coronary artery bypass surgery. Its activity against this enzyme can be assessed using a procedure which is based on a modification of the assay described by Rohrbach, M.S., Anal. Biochem., 1978, 84, 272. The method involves determining the concentration of compound required to reduce by 50% the extent of release of radiolabelled hippuric acid from hippuryl-L-histidyl-L-leucine by angiotensin converting enzyme isolated from the rat kidney.

Inhibitory activity can also be measured in vivo following intravenous injection to anaesthetised rats using the methods described by I.L. Natoff et al, Journal of Pharmacological Methods, 1981, 5, 305 and by D.M. Gross et al, J. Pharmacol, Exp. Ther., 1981, 216, 552. The dose of the inhibitor that is required to reduce the pressor response produced by intravenous injection of angiotensin I (50 ng bolus) by 50% is determined.

The activity of the α-form as a diuretic agent can be determined by measuring its ability to increase urine output and sodium ion excretion in conscious AV-blocked dogs using the methods described by Alabaster, C.T., et al, Brit. J. Pharmacol., 1989, 98, 823P.

The antihypertensive activity of the α-form can be evaluated by measuring the fall in blood pressure following oral or intravenous administration to salt depleted, diuretic primed, spontaneously hypertensive rats, salt depleted renally hypertensive dogs, or desoxycorticosterone acetate (DOCA)/salt hypertensive rats.

For administration to an animal in the treatment of hypertension, congestive heart failure or renal insufficiency, oral dosages of the α-form will generally be in the range of 1-500mg daily, and preferably 5-200mg daily for the treatment of human beings, for an average adult patient. Thus for a typical adult human patient, individual tablets or capsules contain from 1 to 200mg of the compound in a suitable pharmaceutically acceptable diluent or carrier for administration singly, or in multiple doses, once or several times a day. Dosages for intravenous administration would typically be from 0.01 to 50mg, preferably 0.1 to 10mg, of compound per single dose as required. In practice the physician will determine the actual dosage which will be most suitable for an individual patient and it will vary with the age, weight and response of the particular patient. The above dosages are exemplary of the average case but there can, of course, be individual instances where higher or lower dosage ranges are merited, and such are within the scope of this invention.

For human use, the α-form can be administered alone, but will generally be administered in admixture with a pharmaceutically acceptable diluent or carrier selected with regard to the intended route of administration and standard pharmaceutical practice. For example, it may be administered orally in the form of tablets containing such excipients as starch or dibasic calcium phosphate, or in capsules or ovules either alone or in admixture with excipients, or in the form of an elixir or a suspension containing flavouring or colouring agents. It may be injected parenterally, for example, intravenously, intramuscularly or subcutaneously. For parenteral administration, it is best used in the form of a sterile aqueous solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood.

The α-form may be co-administered with other agents that are useful for the control of blood pressure, the treatment of cardiac conditions or renal insufficiency. Thus, for example, it may be co-administered with a cardiac stimulant, for example digitalis, an alpha-blocker, for example doxazosin, a beta-blocker, a calcium channel blocker, for example amlodipine, exogenous ANF, a potassium channel activator or with another diuretic agent as shall be determined by the physician with regard to the particular patient or disease state.

Therapeutic treatment by use of the α-form as disclosed herein can mean curative or prophylactic treatment of a particular disease.

The invention thus further provides:-

(a) a pharmaceutical composition comprising the α-form, γ-form or hydrated δ-form of a compound of the formula (I) together with a pharmaceutically acceptable diluent or carrier.

(b) the α-form, γ-form or hydrated δ-form of a compound of the formula (I), or a pharmaceutical composition thereof, for use as a medicament.

(c) the use of the α-form, γ-form or hydrated δ-form of a compound of the formula (I), or of a pharmaceutical composition thereof, for the manufacture of a medicament for treating a disease which is dependent on the inhibition of angiotensin converting enzyme and/or zinc dependent neutral endopeptidase E.C.3.4.24.11.

(d) use as stated in (c) where the disease is a cardiovascular disorder such as hypertension, congestive heart failure, renal insufficiency or glaucoma.

(e) a method of treatment of an animal, including a human being, to treat a disease which is dependent on the inhibition of angiotensin converting enzyme and/or zinc dependent neutral endopeptidase E.C.3.4.24.11, which comprises administering to said animal a said enzyme and/or said endopeptidase inhibitory amount of the α-form, γ-form or hydrated δ-form of a compound of the formula (I) or a pharmaceutical composition thereof.

(f) a method as stated in (e) where the disease is as stated in (d).

(g) a sodium, potassium, ammonium or (C_1-C_4 alkyl)ammonium salt of a compound of the formula (II).

(h) the γ-form of a compound of the formula (I).

(i) the hydrated δ-form of a compound of the formula (I).

(j) a compound of the formula (XII) with the proviso that P^1 is not benzyloxycarbonyl when P^2, P^3 and P^4 are each t-butyl.

(k) a compound of the formula (XIII) with the proviso that P^5 is not benzyloxycarbonyl.

The preparation of the α-form is illustrated by the following Examples:-

EXAMPLE 1

(S,S,S)-N-(1-[2-Carboxy-3-(N^2-mesyllysylamino)propyl]-1-cyclopentylcarbonyl)tyrosine, α-form

A solution of (S,S,S)-N-(1-[3-(N^6-benzyloxycarbonyl-N^2-mesyllysylamino)-2-carboxypropyl]-1-cyclopentylcarbonyl)tyrosine in ethyl acetate (1190ml) (a portion of the solution obtained according to the method of Preparation 9 and taken to contain 219g of the starting material) was shaken with a solution of sodium hydroxide (23.1g) in water (503ml). The aqueous phase was separated and hydrogenated at 414kPa (60 psi) and room temperature over a 5% palladium-on-carbon catalyst (20g) for 5 hours. The catalyst was then filtered off and the filtrate adjusted to pH 4 with 5N aqueous hydrochloric acid solution and a white solid precipitated. After granulating for 18 hours at room temperature, the solid product was filtered, washed with water and dried to give the title compound as a white solid (124.4g), m.p. 248-250°C. Found: C,53.47; H,7.25; N,9.50. $C_{26}H_{40}N_4O_9S$ requires: C,53.41; H,6.90; N,9.58%.

EXAMPLE 2

(S,S,S)-N-(1-[2-Carboxy-3-(N^2-mesyllysylamino)propyl]-1-cyclopentylcarbonyl)tyrosine, α-form

A solution of (S,S,S)-N-(1-[2-carboxy-3-(N^2-mesyllysylamino)propyl]-1-cyclopentylcarbonyl)tyrosine hydrate (the δ-form, see Preparation 2) (3.0g) in a 1:5 water/methanol mixture (18ml) or a 1:10 water/acetone mixture (33ml) was stirred for 3 days at room temperature. The resulting solid was collected by filtration and dried to give the title compound as a white solid, m.p. 246-8°C (from the aqueous methanol method), m.p. 242-3°C (from the aqueous acetone method).

EXAMPLE 3

(S,S,S)-N-(1-[2-Carboxy-3-(N^2-mesyllysylamino)propyl]-1-cyclopentylcarbonyl)tyrosine, α-form

(S,S,S)-N-(1-[2-Carboxy-3-(N^2-mesyllysylamino)propyl]-1-cyclopentylcarbonyl)tyrosine, γ-form (see Preparations 4, 5, 7 and 8) (0.5g) was dissolved in water (4ml) and methanol (4ml) was added. The resulting solution was stirred for 17 hours at room temperature. A white solid formed which was collected by filtration and dried to give the title compound (0.43g), m.p. 250-252°C.

EXAMPLE 4

(S,S,S)-N-(1-[2-Carboxy-3-(N^2-mesyllysylamino)propyl]-1-cyclopentylcarbonyl)tyrosine, α-form

(S,S,S)-N-(1-[2-Carboxy-3-(N^2-mesyllysylamino)propyl]-1-cyclopentylcarbonyl)tyrosine, β-form (see Preparations 1, 3 and 6) (0.5g) was dissolved in water (4ml) and methanol (4ml) was added. The resulting solution was stirred for 17 hours at room temperature. A white solid formed which was collected by filtration and dried to give the title compound (0.43g), m.p. 249-251°C.

EXAMPLE 5

(S,S,S)-N-(1-[2-Carboxy-3-(N^2-mesyllysylamino)propyl]-1-cyclopentylcarbonyl)tyrosine, α-form

To a solution of the compound of Preparation 12 (2.50g, 3.20 mmol) in 1,4-dioxane (20ml) was added a solution of 1,4-dioxane (20ml) saturated with HCl gas. After 30 minutes, the initially clear solution deposited an oil which was stirred for 24 hours at room temperature. Water (20ml) was added to give a clear solution which was stirred at room temperature for 60

hours. Evaporation of the resulting solution under reduced pressure gave an oil which was dissolved in water and basified with aqueous sodium hydroxide solution until pH 4 was obtained. The solvent was removed by evaporation under reduced pressure and granulation of the resultant material with methanol provided an off-white solid which was collected by filtration and reslurried in water (4ml) overnight. The solids were filtered off and dried to yield the title compound (0.97g), m.p. 225-230°C.
IR and PXRD analysis confirmed the product to be the required α-form.

EXAMPLE 6

(S,S,S)-N-(1-[2-Carboxy-3-(N^2-mesyllysylamino)propyl]-1-cyclopentylcarbonyl)tyrosine, α-form

To a solution of the compound of Preparation 13 (1.78g) in 1,4-dioxane (18ml) was added aqueous 4M hydrochloric acid (18ml). The clear yellow solution was allowed to stir at room temperature for 60 hours followed by an additional 18 hours at 35°C. Removal of the solvent under reduced pressure gave 5.42g of material, 4.22g of which was dissolved in water (10ml), the solution basified with aqueous sodium hydroxide solution to pH 4.0, seeded with the compound of Example 1 and stirred at room temperature for 18 hours. The resulting clear solution was concentrated to about 10ml in volume under reduced pressure, diluted with methanol (15ml) and granulated for 48 hours. The solids were collected by filtration and dried to provide the title compound (1.25g), m.p. 232-235°C.
IR and PXRD analysis confirmed the product to be the required α-form.

EXAMPLE 7

(S,S,S)-N-(1-[2-Carboxy-3-(N^2-mesyllysylamino)propyl]-1-cyclopentylcarbonyl)tyrosine, α-form

To a cooled (10°C) solution of tert-butyl (S,S,S)-N-(1-[3-(N^6-benzyloxycarbonyl-N^2-mesyllysylamino)-2-(tert-butoxycarbonyl)propyl]-1-cyclopentylcarbonyl)-O^4-tert-butyltyrosinate (13.3g, 15.0mmol) in ethyl acetate (27ml) was added a 5.1M solution of hydrogen chloride in ethyl acetate (70ml) (357 mmol of HCl). After 30 minutes the initially clear solution deposited a tar. The mixture was stirred at room temperature for 18 hours. The clear solution was decanted off from the tar and the tar triturated with ethyl acetate (75ml) to give a sticky solid. The decantation and trituration were repeated 5 times to give a hygroscopic solid which was dissolved in water (12ml). The resulting aqueous solution was washed twice with ethyl acetate, basified with aqueous sodium hydroxide solution to pH 4.0, seeded with the compound of Example 1 and stirred at 45-50°C for 42 hours. The off-white solids were collected by filtration, washed with water and acetone and dried to give the title compound (1.95g), m.p. 237-238°C.

IR and PXRD analysis confirmed the product to be the required α-form.

The following Preparations illustrate the preparation of certain intermediate compounds used in synthesising the α-form:-

PREPARATION 1

(S,S,S)-N-(1-[2-Carboxy-3-(N^2-mesyllysylamino)propyl]-1-cyclopentylcarbonyl)tyrosine, β-form

A solution of (S,S,S)-N-(1-[3-(N^6-benzyloxycarbonyl-N^2-mesyllysylamino)-2-carboxypropyl]-1-cyclopentylcarbonyl)tyrosine (see Preparation 9) (371g) in a 9:1 ethanol/water mixture (2.225l) was hydrogenated at 414kPa (60 psi) and room temperature over a 10% palladium-on-carbon catalyst (37.0g) for 4 hours. The catalyst was filtered off and the filtrate evaporated to leave the crude product as a foam. This material was stirred with acetone (3.13l) for 24 hours to give the title compound as a white amorphous solid (283g). Found: C,52.97; H,7.02; N,8.97. $C_{26}H_{40}N_4O_9S$ requires: C,53.41; H,6.90; N,9.58%.

PREPARATION 2

(S,S,S)-N-(1-[2-Carboxy-3-(N^2-mesyllysylamino)propyl]-1-cyclopentylcarbonyl)tyrosine hydrate (the δ-form)

A solution of (S,S,S)-N-(1-[3-(N^6-benzyloxycarbonyl-N^2-mesyllysylamino)-2-carboxypropyl]-1-cyclopentylcarbonyl)tyrosine (see Preparation 9) (351g) in ethyl acetate (1300ml) was added to water (385ml) and the two phase mixture hydrogenated at 414kPa (60 psi) and room temperature over a 5% palladium-on-carbon catalyst (35g) for 20 hours. The catalyst was filtered off, the aqueous phase separated and concentrated to low volume under reduced pressure. The viscous solution was poured into methanol (2.85l) and stirred at room temperature for 18 hours during which time there was a slow precipitation of a solid. The solid was granulated at 5-10°C for 2 hours, filtered, washed with methanol and

dried to give the title compound as a white solid (178.1g), m.p. 168-171°C. Found: C,51.37; H,7.47; N,9.06. $C_{26}H_{40}N_4O_9S.\chi H_2O$ (where $\chi = 1$) requires: C,51.81; H,7.02; N,9.30%.

Water content = 3.6% by weight as determined by Karl Fischer analysis ($\chi = 1$ requires 3.0% by weight).

PREPARATION 3

(S,S,S)-N-(1-[2-Carboxy-3-(N^2-mesyllysylamino)propyl]-1-cyclopentylcarbonyl)tyrosine, ß-form

(S,S,S)-N-(1-[2-Carboxy-3-(N^2-mesyllysylamino)propyl]-1-cyclopentylcarbonyl)tyrosine hydrate (the δ-form, see Preparation 2) (20.0g) was dissolved in water (250ml) at room temperature and the clear solution frozen using a solid carbon dioxide/acetone bath. The solid mass was freeze dried to yield the title compound as a white solid (19.0g). This material decomposed slowly over the temperature range 155-170°C.

PREPARATION 4

(S,S,S)-N-(1-[2-Carboxy-3-(N^2-mesyllysylamino)propyl]-1-cyclopentylcarbonyl)tyrosine, γ-form

(S,S,S)-N-(1-[2-Carboxy-3-(N^2-mesyllysylamino)propyl]-1-cyclopentylcarbonyl)tyrosine hydrate (the δ-form, see Preparation 2) (1.0g) was stirred with either n-propanol or acetonitrile (10ml) for 24 hours at room temperature. In each case the white solid obtained was collected by filtration and dried to provide the title compound, m.p. 172-176°C.

PREPARATION 5

(S,S,S)-N-(1-[2-Carboxy-3-(N^2-mesyllysylamino)propyl]-1-cyclopentylcarbonyl)tyrosine, γ-form

(S,S,S)-N-(1-[2-Carboxy-3-(N^2-mesyllysylamino)propyl]-1-cyclopentylcarbonyl)tyrosine hydrate (the δ-form, see Preparation 2) (847.0g) was dissolved in water (762ml) and the solution diluted with acetone (1.0l). This solution was added slowly to vigorously stirred acetone (18.05l) at room temperature and a white solid precipitated. The mixture was stirred at room temperature for 18 hours, the solid was collected by filtration, washed with acetone and dried to give the title compound as a white solid (775g), m.p. 179-181°C. Found: C,53.42; H,6.88; N,9.37; S,5.49. $C_{26}H_{40}N_4O_9S$ requires: C,53.41; H,6.90; N,9.58; S,5.48%.

PREPARATION 6

(S,S,S)-N-(1-[2-Carboxy-3-(N^2-mesyllysylamino)propyl]-1-cyclopentylcarbonyl)tyrosine, β-form

(S,S,S)-N-(1-[2-Carboxy-3-(N^2-mesyllysylamino)propyl]-1-cyclopentylcarbonyl)tyrosine, α-form (see Examples 1 to 4) (4.0g) was added to water (200ml) and the mixture stirred at 90-95°C for 30 minutes. Insoluble material was filtered off, the filtrate diluted with further water (50ml) and cooled to room temperature. After filtration to remove a slight haze, the clear filtrate was frozen using a solid carbon dioxide/acetone bath. The solid mass obtained was freeze dried to yield the title compound as a white solid (3.0g). This material decomposed slowly over the temperature range 155-165°C.

PREPARATION 7

(S,S,S)-N-(1-[2-Carboxy-3-(N^2-mesyllysylamino)propyl]-1-cyclopentylcarbonyl)tyrosine, γ-form

(S,S,S)-N-(1-[2-Carboxy-3-(N^2-mesyllysylamino)propyl]-1-cyclopentylcarbonyl)tyrosine, ß-form (see Preparations 1, 3 and 6) (0.3g) was slurried in acetonitrile (15ml) and stirred for 5 days. The resulting white solid was collected by filtration and dried under reduced pressure to provide the title compound (0.26g).

PREPARATION 8

(S,S,S)-N-(1-[2-Carboxy-3-(N^2-mesyllysylamino)propyl)-1-cyclopentylcarbonyl)tyrosine, γ-form

(S,S,S)-N-(1-[2-Carboxy-3-(N^2-mesyllysylamino)propyl)-1-cyclopentylcarbonyl)tyrosine, ß-form (see Preparations 1, 3 and 6) (0.3g) was slurried in n-propanol (10ml) and stirred for 5 days. The resulting white solid was collected by filtration and dried under reduced pressure to provide the title compound (0.26g), m.p. 175-180°C.

PREPARATION 9

(S,S,S)-N-(1-[3-(N^6-Benzyloxycarbonyl-N^2-mesyllysylamino)-2-carboxypropyl]-1-cyclopentylcarbonyl)tyrosine

Tert-butyl (S,S,S)-N-(1-[3-(N^6-benzyloxycarbonyl-N^2-mesyllysylamino)-2-(tert-butoxycarbonyl)propyl]-1-cyclopentylcarbonyl)-O^4-tert-butyltyrosinate (404g) was dissolved in dichloromethane (810ml). Anisole (769g) was added in one portion and then trifluoroacetic acid (1.158kg) added dropwise over approximately 10 minutes. On completion of the addition, the reaction was stirred at 35°C for 6 hours and then stirred at room temperature overnight. Water (1000ml) was added and three layers formed. The top and bottom layers were combined, dissolved in ethyl acetate (2l) and the resulting solution washed with brine. The organic

phase was mixed with brine, the pH adjusted to 3 and the layers allowed to separate. Three layers formed. The organic phases were separated, taken up in ethyl acetate and extracted with saturated aqueous sodium bicarbonate (1.6l) solution and brine (0.5l). The combined aqueous layers were washed with ethyl acetate, then acidified and extracted with ethyl acetate to give an ethyl acetate solution (1.54l) of the title compound. This solution was either used directly (e.g. see Example 1) or the solvent removed to provide the title compound.

PREPARATION 10

(S)-N^6-Benzyloxycarbonyl-N^2-mesyllysine

(S)-N^6-Benzyloxycarbonyllysine (1.5kg) was slurried in methylene chloride (7.5l) and chlorotrimethylsilane (1.36l) added over 10 minutes. The mixture was heated under reflux for 30 minutes to give a solution which was cooled to 3°C before simultaneously adding diisopropylethylamine (1.87l) and methanesulphonyl chloride (435ml) at such a rate as to keep the temperature below 25°C. The reaction was stirred for a further 2.5 hours then poured into 2 M aqueous hydrochloric acid solution. The layers were separated and the methylene chloride phase was washed with 2 M aqueous hydrochloric acid solution followed by water. The solvent was removed under reduced pressure and replaced with n-butyl acetate. The solution was cooled and the resulting crystalline material was collected by filtration, washed with n-butyl acetate and dried under reduced pressure to provide the title compound (1.63kg), m.p. 83.5-84°C. $[\alpha]^{25}_{D}$ -13.4° (c = 1, methanol). Found: C,50.23; H,6.40; N,7.76.

$C_{15}H_{22}N_2O_6S$ requires: C,50.27; H,6.19; N,7.82%.

^{1}H-NMR (300MHz, d_6-DMSO): δ = 1.23-1.78(6H,m), 2.85(3H,s), 2.98(2H,q), 3.80(1H,dt), 5.00(2H,s), 7.25(1H,t), 7.30-7.43(5H,m), 7.51(1H,d) ppm.

PREPARATION 11

Tert-butyl (S,S,S)-N-(1-[2-tert-butoxycarbonyl-3-(N^2-mesyllysylamino)propyl]-1-cyclopentylcarbonyl)-O^4-tert-butyltyrosinate

To a solution of tert-butyl (S,S,S)-N-(1-[3-(N^6-benzyloxycarbonyl-N^2-mesyllysylamino)-2-(tert-butoxycarbonyl)propyl]-1-cyclopentylcarbonyl)-O^4-tert-butyltyrosinate (48.64g, 54.8 mmmol) in industrial methylated spirits (1.0L) was added 5% palladium-on-carbon (5g) (water wet) and the mixture was hydrogenated at 345-414kPa (50-60 psi) and at room temperature for 19 hours. After removal of the catalyst by filtration, the resulting solution was concentrated under reduced pressure to provide the title compound as a colourless oil (46.56g) which contained ethanol.

^{1}H-NMR (300MHz, $CDCl_3$): δ = 1.27(9H,s), 1.41(9H,s), 1.44(9H,s), 1.45-1.62(14H, broad m), 1.8-2.05(4H, broad m), 2.21(2H,m), 2.72(2H,t), 2.79(3H, broad), 2.96(3H,s), 3.1(2H,m), 3.59(1H,m), 3.96(1H,t), 4.73(1H,m), 6.43(1H,dt), 6.89(2H,dt), 7.09(2H,dt), 7.51(1H,dt) ppm.

PREPARATION 12

Tert-butyl (S,S,S)-N-(1-[2-tert-butoxycarbonyl-3-(N^6-formyl-N^2-mesyllysylamino)propyl]-1-cyclopentylcarbonyl)-O^4-tert-butyltyrosinate

A cooled (0°C) solution of formic acetic anhydride in acetic acid (made by combining 45.3ml of acetic anhydride with 22.8ml of formic acid, heating the resulting solution to 50-60°C for 15 minutes, then cooling to 0°C) was added to a solution of the compound of Preparation 11 (27.3g, 36.3 mmol) in formic acid (33.7ml) at 0°C over 10 minutes. The solution was allowed to warm to and stirred at room temperature for 45 minutes and then quenched onto ice. The resulting mixture was neutralised with aqueous sodium hydroxide solution and extracted with dichloromethane (x 2). The combined organic layers were washed twice with brine and evaporated under reduced pressure to provide the title compound as a yellow foam (28.0g).

^{1}H-NMR (300MHz, $CDCl_3$): δ = 1.26(9H,s), 1.41(18H,s), 1.45-2.03(16H, broad), 2.23(2H, broad m), 2.97(3H,s), 3.08(2H,m), 3.28(2H,m), 3.51(1H,m), 3.98(1H, broad m), 4.73(1H,q), 5.57(1H, broad dt), 5.91(1H, broad), 6.32(1H,dt), 6.90(2H,dt), 7.08(2H,dt), 7.29(1H,broad), 8.17(1H,s) ppm.

PREPARATION 13

(S,S,S)-N-(1-[2-Carboxy-3-(N^6-formyl-N^2-mesyllysylamino)propyl]-1-cyclopentylcarbonyl)tyrosine

To a cooled (0°C) solution of the compound of Preparation 12 (2.71g, 3.46 mmol) in dichloromethane (4.8ml) was added trifluoroacetic acid (4.8ml). The reaction was allowed to warm to room temperature and stirred for 24 hours. The mixture was then concentrated under reduced pressure to provide the title compound as a solid (2.4g), m.p. 56-60°C. ^{1}H-NMR (300MHz, d_6-DMSO): δ = 1.2-1.6(14H, broad m), 1.71-1.86(3H,m), 1.86-1.99(1H,m), 2.28-2.41(1H,m), 2.78(3H,s), 2.8-3.09(4H,m), 3.12-3.25(2H,m), 3.7(1H,m), 4.35(1H,m), 6.6(2H,dt), 6.98(2H,dt), 7.25(1H,dt), 7.50(1H,dt), 7.91(2H,m), 7.97(1H,s) ppm.

Characterisation of the α-, β-, γ- and δ-forms by IR, PXRD and DSC analysis and by melting point determination

a) Infra-red spectroscopy (IR)

The infra-red spectra of the different forms were determined as nujol mulls using a Nicolet 800 FT-IR spectrometer. For each form, the wave numbers (ν [cm^{-1}]) of the absorption bands are listed in Table 1.

TABLE 1

α-form	β-form	γ-form	δ-form
			3667*
3407*			3425*
3386*	3384	3377	3380
3223		3240	3287
3153			3137
			3098
	1708		1709
1699			
			1673*
1652*		1665*	
	1638	1639	1637
1626			
	1615		1619
1594	1595	1594	1596
			1568
			1556
	1533		
		1527	
1516	1516	1518	1516
		1494*	
1457 (nujol)	1458 (nujol)	1457 (nujol)	1458 (nujol)
		1443	1448
			1419
	1396		1390
1377 (nujol)	1378 (nujol)	1377 (nujol)	1378 (nujol)
			1356
1344		1344	
1334			1338
		1321	
1317	1313		
		1304	1300
			1270
1267			
		1254	
1241	1245		1249
1228			1229
1210			

TABLE 1 (continued)

α-form	β-form	γ-form	δ-form
		1195	1198
	1172	1178	1174
1164		1162	
1151*			
	1144	1143	1141
1137			
1118		1111	
1109	1106		1108
1093		1098	1091
1074			1075
			1064
1045		1046	1045
		1031	1033
1019		1012	1019
1003			1001
981	980		985
		972	
965		962	962
		945	941
		932	
911			
		907	909
897*			
	889		889
		879	877
862			
		849	841
	830		
			822
818		815	
800	808	806	807
		780	
778			
762			763
		753	
			744
	737		732
721	721	729	721
	665		
655		658	655

* indicates those bands which are considered to be the most significant in terms of differentiating between the various forms.

Representative infra-red spectra for the various forms are shown in Figures 1A, 1B, 2A, 2B, 3A, 3B, 4A and 4B.

(b) Powder X-ray diffraction (PXRD)

The powder X-ray diffraction patterns of the various forms were obtained using a Siemens D500 diffractometer that was operated at 40kV/30mA and using copper radiation filtered with a graphite monochromator (λ = 0.15405nm) and a scintillation counter detector. For each form, beam intensity as a function of the angle 2θ was recorded over the range 2° to 45° 2θ using a step scan mode counting for six seconds at step intervals of 0.03° 2θ. For each form, the main peaks (degrees 2θ) seen in the pattern are listed in Table 2.

TABLE 2

α-form (sharp peaks)	β-form	γ-form (sharp peaks)	δ-form (sharp peaks)
7.5			
8.9			
9.9	Broad peaks with centres at 11 and 20	9.0, 9.6	
		10.6	10.5, 10.8
11.6		11.6	
		12.7	12.3
		13.3	
		14.6	14.5
15.6			
		16.2	
17.2, 17.5		17.9	17.2, 17.6, 17.9
18.0		18.8	18.9
20.2		20.2	20.4
		21.8	21.5
22.1			22.4
23.3			23.0, 23.1
			24.7
			27.1, 27.8
			28.9

Representative powder X-ray diffraction patterns for the various forms are shown in Figures 5 to 8.

(c) <u>Differential Scanning Calorimetry</u> (DSC)

Samples (about 5mg) of the various forms were analysed using a Perkin-Elmer 7 Series thermal analyser at a scanning rate of 20°C per minute. The results obtained for the various forms are summarised in Table 3.

TABLE 3

Form	Summary of DSC analysis
α-form	Sharp endotherm in the range 248-259°C. Decomposition above 260°C.
ß-form	Broad endotherm in the range 60-130°C. Weak endotherm at about 147°C. Decomposition above 200°C.
γ-form	Sharp endotherm in the range 176-186°C. Sharp exotherm at about 207°C. Weak endotherm at about 213°C. Decomposition above 250°C.
δ-form	Sharp endotherms at about 162 and at about 166-168°C. Decomposition above 200°C.

Representative DSC thermograms for the various forms are shown in Figures 9 to 12.

(d) Melting point

The melting points of the various forms were determined by hot stage microscopy using a Mettler FP5/FP52 apparatus at a heating rate of 2°C per minute. The typical ranges within which the various forms melt are set out in Table 4.

TABLE 4

Form	Sharp melting points in the range (°C)
α-form	242-252
γ-form	170-185
δ-form	165-175

Comparative studies

The α- and β-forms were compared using processing and hygroscopicity studies.

(a) Processing study

An instrumented tablet machine (Manesty Machines Limited, Model F3) was satisfactorily calibrated for force and upper punch displacement.

When calibrated, a placebo Avicel (trade mark)/DCP (dibasic calcium phosphate) blend was processed on the machine using 13mm flat faced punches to measure the reproducibility of the technique. Using an aliquot of the blend, the machine was adjusted appropriately to achieve the target compression weight (400mg) and sufficient hardness. Twenty unit aliquots were then separately weighed and loaded into the shoe of the machine. The machine was operated under power until the blend in the shoe had been exhausted and no further tablets were produced. Figure 13 shows a plot of upper punch force as a function of the number of tablets for three Avicel/DCP placebo blends, each of twenty units, and Table 5 shows the mean weight and hardness of the ten heaviest tablets (assumed to be the first ten produced). It can be seen from the data presented in Figure 13 that the overall process, for this blend, was very reproducible. The decrease in upper punch force that occurred at the end of the run can be correlated with the reduction in the amount of blend in the shoe and consequential poor filling of the die.

TABLE 5

Table showing the mean weight and hardness of tablets produced using an Avicel/DCP placebo blend.

Run	Mean weight (mg)	Standard Deviation	Mean hardness (kPa)	Standard Deviation
1	394.3	7.82	16.0	1.68
2	389.6	9.20	14.6	2.04
3	393.3	6.93	15.1	1.22

Following the experiment to determine the reproducibility of the technique, blends containing the α-form or the ß-form were separately prepared according to the following formulation: α- or ß-form (100mg), pregelatinsed starch (40mg), dibasic calcium phosphate (anhydrous grade) (256mg) and magnesium stearate (2mg). A blend/screen/blend process was used to manufacture 20g of the blend prior to slugging on the machine. The loading was 100mg as previous experience had indicated that the higher the loading, the more processing difficulties that were encountered. The machine was adjusted for the blend and then 50 tablets were produced from the particular blend in one continuous batch.

Optimisation of the machine was more difficult with the ß-form blend due to its poor flow properties. Despite careful manipulation of the process variables, it was not possible to maintain the upper punch force constant between both blends and consequently the ß-form blend was compressed to a greater hardness.

The upper punch data are shown for both blends in Figure 14. The large variability in upper punch force (and tablet weight) for the ß-form blend was associated with the non-uniform filling of the die for this formulation. The data presented in Table 6 confirms that processing of the ß-form formulation was much more difficult and was subject to much greater variability than if the α-form formulation was used.

TABLE 6

Table showing the variability in processing parameters for blends containing the α- and ß-forms.

Sample	Mean upper punch force (kN)	Standard Deviation	Mean tablet weight (mg)	Standard Deviation	Mean hardness (kP)	Standard Deviation
α-form blend	18.0	1.85	398	17.6	5.0	1.15
ß-form blend	23.2	7.07	446	48.7	18.5	4.69

The measured ejection force for the last ten tablets of each blend is shown in Figure 15. The tablets formed from the ß-form required much greater force to remove them from the die. This effect manifested itself in the tablets being "flipped" from the die by the shoe.

The data obtained shows the poor processing properties of the β-form as compared to the α-form. The β-form has a low bulk density (fluff density = 0.09g ml^{-1}, compared with 0.36g ml^{-1} for the α-form) and poor flow properties and when blends containing it are tabletted, a large variability in tablet weight results and a high ejection force is required. In all these respects, the α-form has been shown to exhibit superior properties making it particularly suitable for pharmaceutical formulation.

(b) Hygroscopicity study

(i) The hygroscopicity of the α- and β-forms was assessed by gravimetric analysis as follows.

Samples of the α- and β-forms were separately placed in Kilner (trade mark) jars under the following conditions: 40°C; 40°C and 75%RH (relative humidity); and 40°C and 95%RH. Water uptake of each sample was assessed gravimetrically, in triplicate, after selected time intervals.

Samples of the β-form stored at 40°C/75%RH or 40°C/95%RH for 1 day underwent a morphological change. Samples of the β-form stored at 40°C/95%RH for 1 day underwent a small weight loss (presumably after a weight increase due to water absorption followed by the morphological change and then moisture loss), whereas samples stored at 40°C/75%RH gained, on average, 6% of their original weight.

Figure 16 shows the results obtained from the gravimetric analysis. The α-form was not found to be hygroscopic. However the β-form was found to be very hygroscopic at 40°C/75%RH.

(ii) Moisture microbalance experiments on the α- and ß-forms confirmed that the α-form was not hygroscopic whereas the ß-form was very hygroscopic.

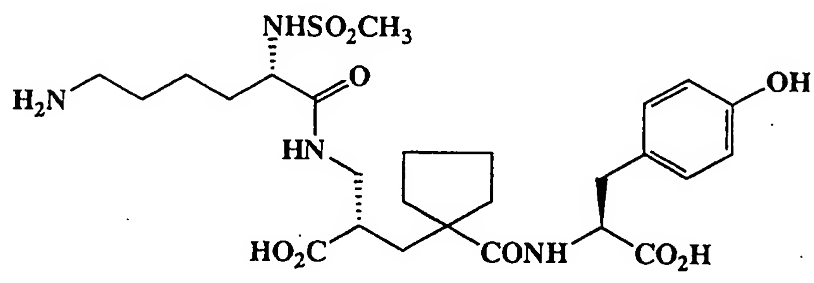
Samples of the α- and ß-forms were separately placed in the apparatus at 40°C and allowed to equilibrate with the surroundings prior to the particular sample being exposed to increasing relative humidities, with equilibration periods between each increase in humidity.

The results are shown in Figure 17. These indicate that as much as 8% by weight of water (cf. original weight) was taken up by the ß-form during the experiment.

The morphological change that the ß-form underwent at high humidities was further studied and a transformation from a very low bulk density powder to a dense glassy solid was observed.

CLAIMS

1. A crystalline, α-polymorphic form of a compound of the formula:-



,

(I)

characterised by an infra-red spectrum as a mull in nujol which shows absorption bands at ν = 3407, 3386, 3223, 3153, 1699, 1652, 1626, 1594, 1516, 1457 (nujol), 1377 (nujol), 1344, 1334, 1317, 1267, 1241, 1228, 1210, 1164, 1151, 1137, 1118, 1109, 1093, 1074, 1045, 1019, 1003, 981, 965, 911, 897, 862, 818, 800, 778, 762, 721 and 655 cm^{-1}.

2. A compound as claimed in claim 1 which is further characterised by a powder X-ray diffraction pattern obtained using copper radiation filtered with a graphite monochromator (λ = 0.15405nm) which shows main peaks at 7.5, 8.9, 9.9, 11.6, 15.6, 17.2, 17.5, 18.0, 20.2, 22.1 and 23.3 degrees 2θ.

3. A γ-polymorphic form of a compound of the formula (I) as defined in claim 1 characterised by an infra-red spectrum as a mull in nujol which shows absorption bands at ν = 3377, 3240, 1665, 1639, 1594, 1527, 1518, 1494, 1457 (nujol), 1443, 1377 (nujol), 1344, 1321, 1304, 1254, 1195, 1178, 1162, 1143, 1111, 1098, 1046, 1031, 1012, 972, 962, 945, 932, 907, 879, 849, 815, 806, 780, 753, 729 and 658 cm^{-1}.

4. A compound as claimed in claim 3 which is further characterised by a powder X-ray diffraction pattern obtained using copper radiation filtered with a graphite monochromator (λ = 0.15405nm) which shows main peaks at 9.0, 9.6, 10.6, 11.6, 12.7, 13.3, 14.6, 16.2, 17.9, 18.8, 20.2 and 21.8 degrees 2θ.

5. A hydrated δ-form of a compound of the formula (I) as defined in claim 1 characterised by a water content of from 1 to 7% by weight, as determined by Karl Fischer analysis, and an infra-red spectrum as a mull in nujol which shows absorption bands at ν = 3667, 3425, 3380, 3287, 3137, 3098, 1709, 1673, 1637, 1619, 1596, 1568, 1556, 1516, 1458 (nujol), 1448, 1419, 1390, 1378 (nujol), 1356, 1338, 1300, 1270, 1249, 1229, 1198, 1174, 1141, 1108, 1091, 1075, 1064, 1045, 1033, 1019, 1001, 985, 962, 941, 909, 889, 877, 841, 822, 807, 763, 744, 732, 721 and 655 cm^{-1}.

6. A compound as claimed in claim 5 which is further characterised by a powder X-ray diffraction pattern obtained using copper radiation filtered with a graphite monochromator (λ = 0.15405nm) which shows main peaks at 10.5, 10.8, 12.3, 14.5, 17.2, 17.6, 17.9, 18.9, 20.4, 21.5, 22.4, 23.0, 23.1, 24.7, 27.1, 27.8 and 28.9 degrees 2θ.

7. A compound as claimed in claim 5 or 6 which has a water content of from 2 to 4% by weight, as determined by Karl Fischer analysis.

8. A pharmaceutical composition comprising the α-polymorphic form of a compound of the formula (I) as claimed in claim 1 or 2, the γ-polymorphic form of a compound of the formula (I) as claimed in claim 3 or 4, or the hydrated δ-form of a compound of the formula (I) as claimed in claim 5, 6 or 7, together with a pharmaceutically acceptable diluent or carrier.

9. A composition as claimed in claim 8 wherein the α-polymorphic form of a compound of the formula (I) is present.

10. The α-polymorphic form of a compound of the formula (I) as claimed in claim 1 or 2, the γ-polymorphic form of a compound of the formula (I) as claimed in claim 3 or 4, or the hydrated δ-form of a compound of the formula (I) as claimed in claim 5, 6 or 7, or a pharmaceutical composition of any thereof, as claimed in claim 8 or 9, as appropriate, for use as a medicament.

11. A compound or composition thereof for use as a medicament as claimed in claim 10 wherein the compound is the α-polymorphic form of a compound of the formula (I).

12. The use of the α-polymorphic form of a compound of the formula (I) as claimed in claim 1 or 2, the γ-polymorphic form of a compound of the formula (I) as claimed in claim 3 or 4, or the hydrated δ-form of a compound of the formula (I) as claimed in claim 5, 6 or 7, or of a pharmaceutical composition of any thereof as claimed in claim 8 or 9, as appropriate, for the manufacture of a medicament for treating a disease which is dependent on the inhibition of angiotensin converting enzyme and/or zinc dependent neutral endopeptidase E.C.3.4.24.11.

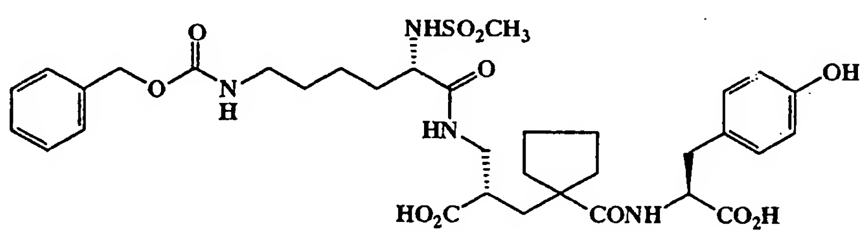
13. The use as claimed in claim 12 wherein the disease is a cardiovascular disorder such as hypertension, congestive heart failure, renal insufficiency or glaucoma.

14. The use as claimed in claim 12 or 13 wherein the α-polymorphic form of a compound of the formula (I) or a composition thereof is used.

15. A method of treatment of an animal, including a human being, to treat a disease which is dependent on the inhibition of angiotensin converting enzyme and/or zinc dependent neutral endopeptidase E.C.3.4.24.11 such as a cardiovascular disorder, for example, hypertension, congestive heart failure, renal insufficiency or glaucoma, which comprises administering to said animal a said enzyme and/or said endopeptidase inhibitory amount of the α-polymorphic form of a compound of the formula (I) as claimed in claim 1 or 2, the γ-polymorphic form of a compound of the formula (I) as claimed in claim 3 or 4, or the hydrated δ-form of a compound of the formula (I) as claimed in claim 5, 6 or 7, or a pharmaceutical composition of any thereof as claimed in claim 8 or 9, as appropriate.

16. A method as claimed in claim 15 wherein the α-polymorphic form of a compound of the formula (I) or a composition thereof is used.

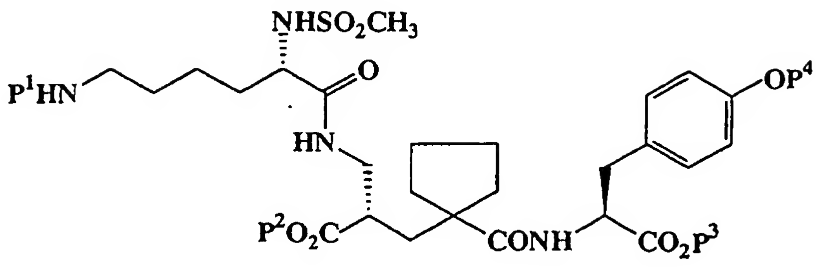
17. A sodium, potassium, ammonium or (C_1-C_4 alkyl)ammonium salt of a compound of the formula:-



(II)

18. A sodium salt of a compound of the formula (II) as claimed in claim 17.

19. A compound of the formula:



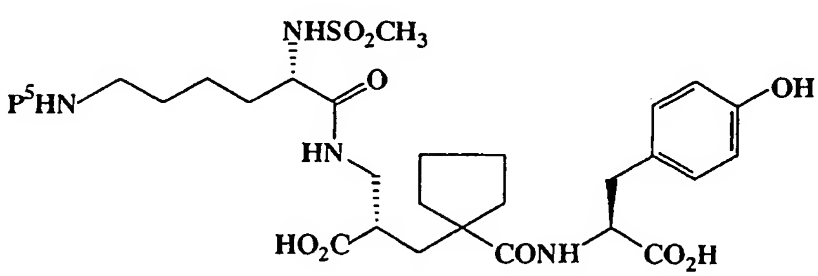
(XII)

wherein P^1, P^2, P^3 and P^4, which may be the same or different, are all protecting groups that are capable of removal, preferably under acidic conditions, to provide a compound of the formula (I) as defined in claim 1, with the proviso that P^1 is not benzyloxycarbonyl when P^2, P^3 and P^4 are each t-butyl.

20. A compound as claimed in claim 19 wherein P^1 is formyl or benzyloxycarbonyl.

21. A compound as claimed in claim 19 or 20, in part, wherein P^2, P^3 and P^4 are each t-butyl.

22. A compound of the formula:

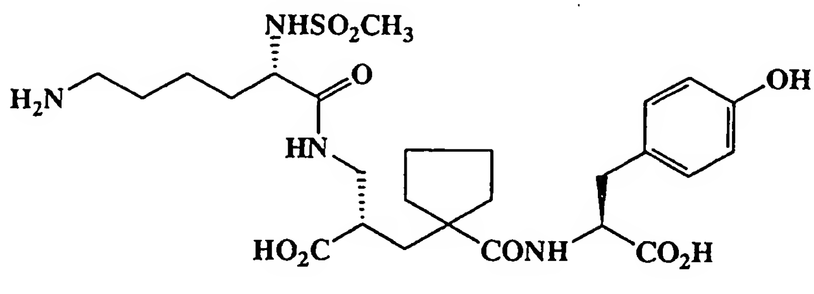


(XIII)

wherein P^5 is a protecting group that is capable of removal, preferably under acidic conditions, to provide a compound of the formula (I) as defined in claim 1, with the proviso that P^5 is not benzyloxycarbonyl.

23 A compound as claimed in claim 22 wherein P^5 is formyl.

24. A process for the preparation of a crystalline, α-polymorphic form of a compound of the formula:-

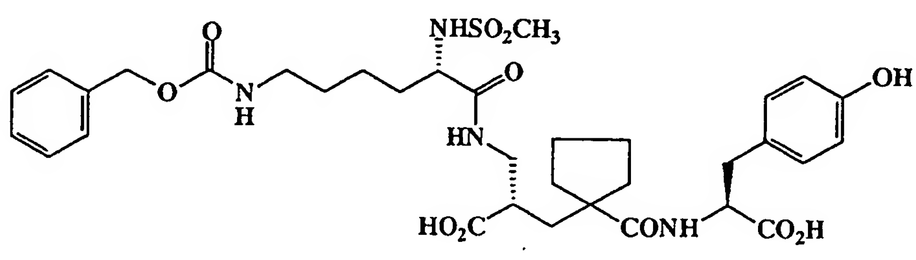


,

(I)

that is characterised by an infra-red spectrum as a mull in nujol which shows absorption bands at ν = 3407, 3386, 3223, 3153, 1699, 1652, 1626, 1594, 1516, 1457 (nujol), 1377 (nujol), 1344, 1334, 1317, 1267, 1241, 1228, 1210, 1164, 1151, 1137, 1118, 1109, 1093, 1074, 1045, 1019, 1003, 981, 965, 911, 897, 862, 818, 800, 778, 762, 721 and 655 cm^{-1}, which comprises

a) catalytic hydrogenation of an aqueous solution of a sodium, potassium, ammonium or (C_1-C_4 alkyl)ammonium salt of a compound of the formula:-



(II)

using a catalyst capable of the removal of the benzyloxycarbonyl protecting group, followed by acidification of the base salt of the compound of the formula (I) obtained to from pH 3 to 5, preferably about pH 4, to provide the required α-polymorphic form;

(b) treatment of a hydrated δ-form of a compound of the formula (I) as defined in this claim characterised by a water content of from 1 to 7% by weight, as determined by Karl Fischer analysis, and an infra-red spectrum as a mull in nujol which shows absorption bands at ν = 3667, 3425, 3380, 3287, 3137, 3098, 1709, 1673, 1637, 1619, 1596, 1568, 1556, 1516, 1458 (nujol), 1448, 1419, 1390, 1378 (nujol), 1356, 1338, 1300, 1270, 1249, 1229, 1198, 1174, 1141, 1108, 1091, 1075, 1064, 1045, 1033, 1019, 1001, 985, 962, 941, 909, 889, 877, 841, 822, 807, 763, 744, 732, 721 and 655 cm^{-1}, with water, an aqueous solution of a C_1-C_4 alkanol such as methanol or 2-propanol, or an aqueous solution of a C_3-C_6 alkanone such as acetone;

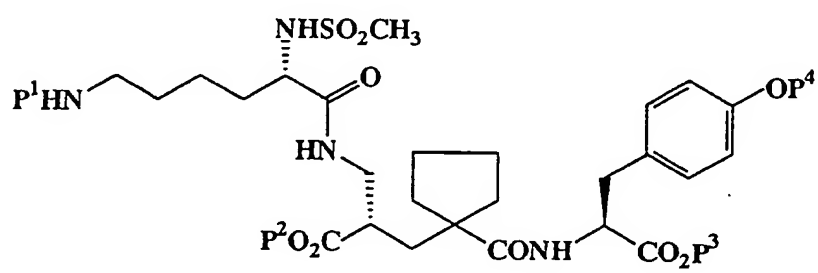
(c) treatment of a γ-polymorphic form of a compound of the formula (I) as defined in this claim that is characterised by an infra-red spectrum as a mull in nujol which shows absorption bands at ν = 3377, 3240, 1665, 1639, 1594, 1527, 1518, 1494, 1457 (nujol), 1443, 1377 (nujol), 1344, 1321, 1304, 1254, 1195, 1178, 1162, 1143, 1111, 1098, 1046, 1031, 1012, 972, 962, 945, 932, 907, 879, 849, 815, 806, 780, 753, 729 and 658 cm^{-1}, with water, an aqueous solution of a C_1-C_4 alkanol such as methanol or 2-propanol, or an aqueous solution of a C_3-C_6 alkanone such as acetone;

(d) treatment of an amorphous β-form of a compound of the formula (I) as defined in this claim that is characterised by an infra-red spectrum as a mull in nujol which shows

absorption bands at ν = 3384, 1708, 1638, 1615, 1595, 1533, 1516, 1458 (nujol), 1396, 1378 (nujol), 1313, 1245, 1172, 1144, 1106, 980, 889, 830, 808, 737, 721 and $665cm^{-1}$, with water, an aqueous solution of a C_1-C_4 alkanol such as methanol or 2-propanol, or an aqueous solution of a C_3-C_6 alkanone such as acetone;

(e) catalytic hydrogenation of a solution of a compound of the formula (II) as defined in this claim in an aqueous organic solvent in the presence of a catalyst capable of the removal of the benzyloxycarbonyl protecting group, removal of the catalyst and the solvent from the mixture on essential completion of the removal of the benzyloxycarbonyl protecting group and stirring of the residue with a C_3-C_6 alkanone such as acetone;

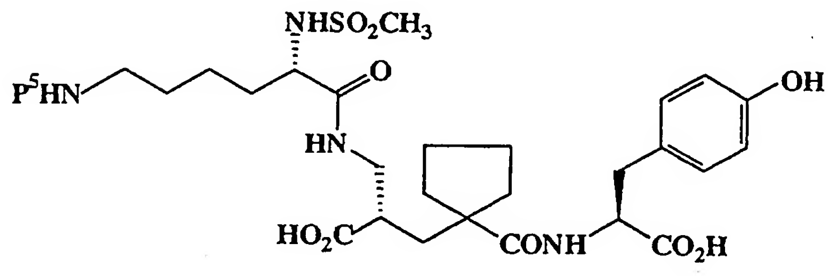
(f) deprotection, preferably under acidic conditions, of a compound of the formula:



(XII)

wherein P^1, P^2, P^3 and P^4, which may be the same or different, are all protecting groups that are capable of removal, preferably under acidic conditions, to provide, following adjustment of the pH to from 3 to 5, preferably about 4, in the work-up, the required α-polymorphic form; or

(g) deprotection, preferably under acidic conditions, of a compound of the formula:



(XIII)

wherein P^5 is a protecting group that is capable of removal, preferably under acidic conditions, to provide, following adjustment of the pH to from 3 to 5, preferably about 4, in the work-up, the required α-polymorphic form.

25. A process as claimed in claim 24(a) wherein the catalyst is palladium-on-carbon.

26. A process as claimed in claim 24(a) or 25 wherein a sodium salt of a compound of the formula (II) is used.

27. A process as claimed in claim 24(a), 25 or 26 wherein the acidification is carried out at from 35 to 45°C.

28. A process as claimed in claim 24(f) wherein P^1 is formyl or benzyloxycarbonyl.

29. A process as claimed in claim 24(f) or 28 wherein P^2, P^3 and P^4 are each t-butyl.

30. A process as claimed in claim 24(g) wherein P^5 is formyl.

31. A process as claimed in any of claims 24 to 30 wherein the α-polymorphic form of a compound of the formula (I) is further characterised by a powder X-ray diffraction pattern obtained using copper radiation filtered with a graphite monochromator (λ = 0.15405nm) which shows main peaks at 7.5, 8.9, 9.9, 11.6, 15.6, 17.2, 17.5, 18.0, 20.2, 22.1 and 23.3 degrees 2θ.

32. A process for the preparation of the γ-polymorphic form of a compound of the formula (I) as defined in claim 24(c) which comprises treatment of the hydrated δ-form of a compound of the formula (I) as defined in claim 24(b), with 1-propanol or acetonitrile.

33. A process for the preparation of the γ-polymorphic form of a compound of the formula (I) as defined in claim 24(c) which comprises treatment of an amorphous β-form of a compound of the formula (I) as defined in claim 24(d), with acetonitrile or n-propanol.

34. A process for the preparation of the γ-polymorphic form of a compound of the formula (I) as defined in claim 24(c) which comprises treatment of an aqueous solution of the hydrated δ-form of a compound of the formula (I) as defined in claim 24(b), with a volumetric excess of a C_3-C_6 alkanone such as acetone.

35. A process for the preparation of the hydrated δ-form of a compound of the formula (I) as defined in claim 24(b) which comprises catalytic hydrogenation of a solution of a compound of the formula (II) as defined in claim 24(a) in a mixture of a water-immiscible solvent and water and in the presence of a catalyst capable of the removal of the benzyloxycarbonyl protecting group, removal of the catalyst and separation of the aqueous layer on essential completion of the removal of the benzyloxycarbonyl protecting group, followed by treatment of the aqueous layer with a C_1-C_4 alkanol such as methanol.

36. A process for the preparation of an amorphous β-form of a compound of the formula (I) as defined in claim 24(d) which comprises freeze drying a frozen aqueous solution of the hydrated δ-form of a compound of the formula (I) as defined in claim 24(b).

37. A process for the preparation of an amorphous β-form of a compound of the formula (I) as defined in claim 24(d) which comprises freeze drying a concentrated aqueous solution of the α-polymorphic form of a compound of the formula (I) as defined in claim 24.

FIGURE 1A

IR spectrum of the α-form as a nujol mull on a sodium chloride disc with air as the reference (ν = 4000-400cm^{-1})

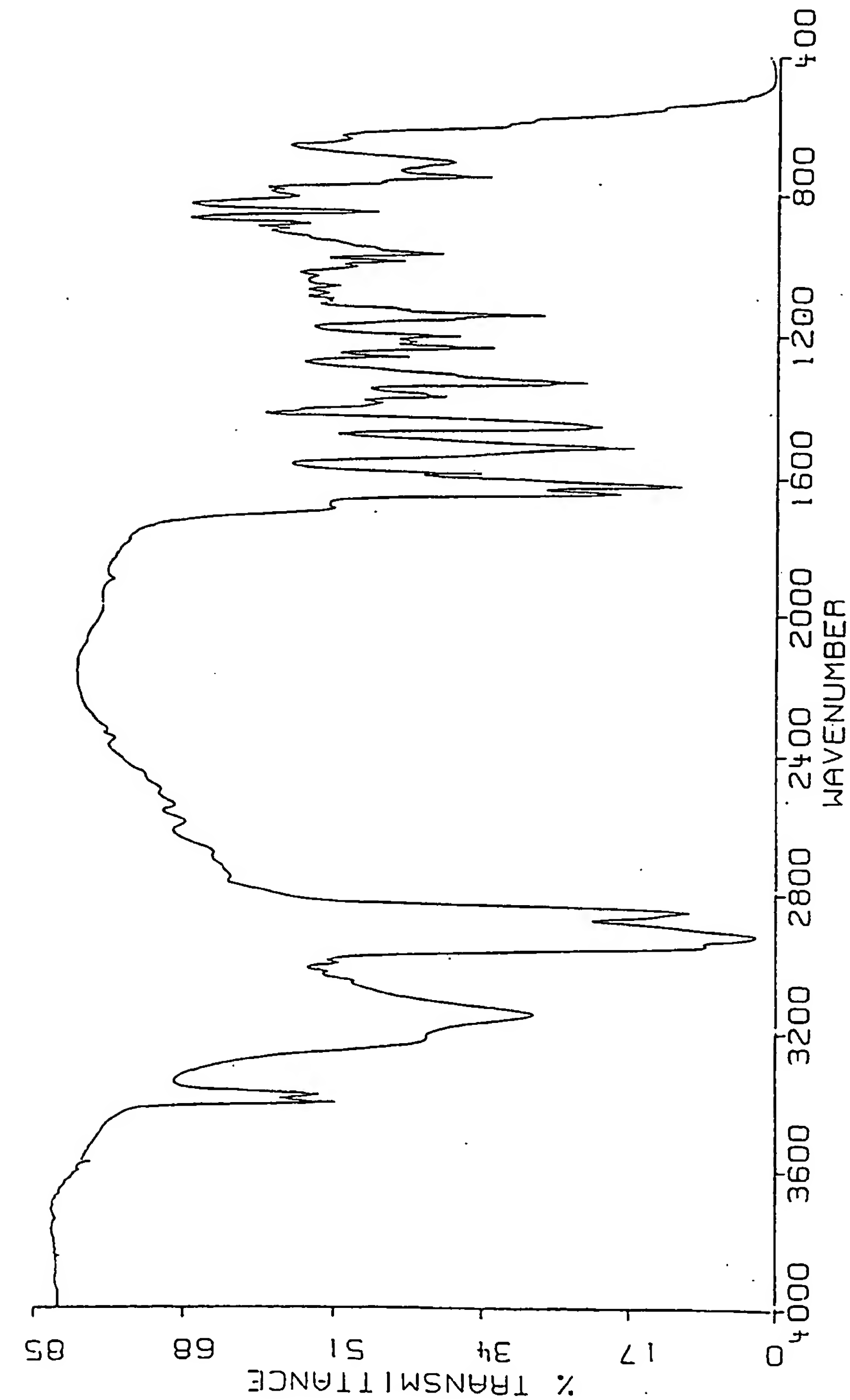


FIGURE 1B

IR spectrum of the α-form as a nujol mull on a sodium chloride disc with air as the reference
(ν = 2000-400cm^{-1})

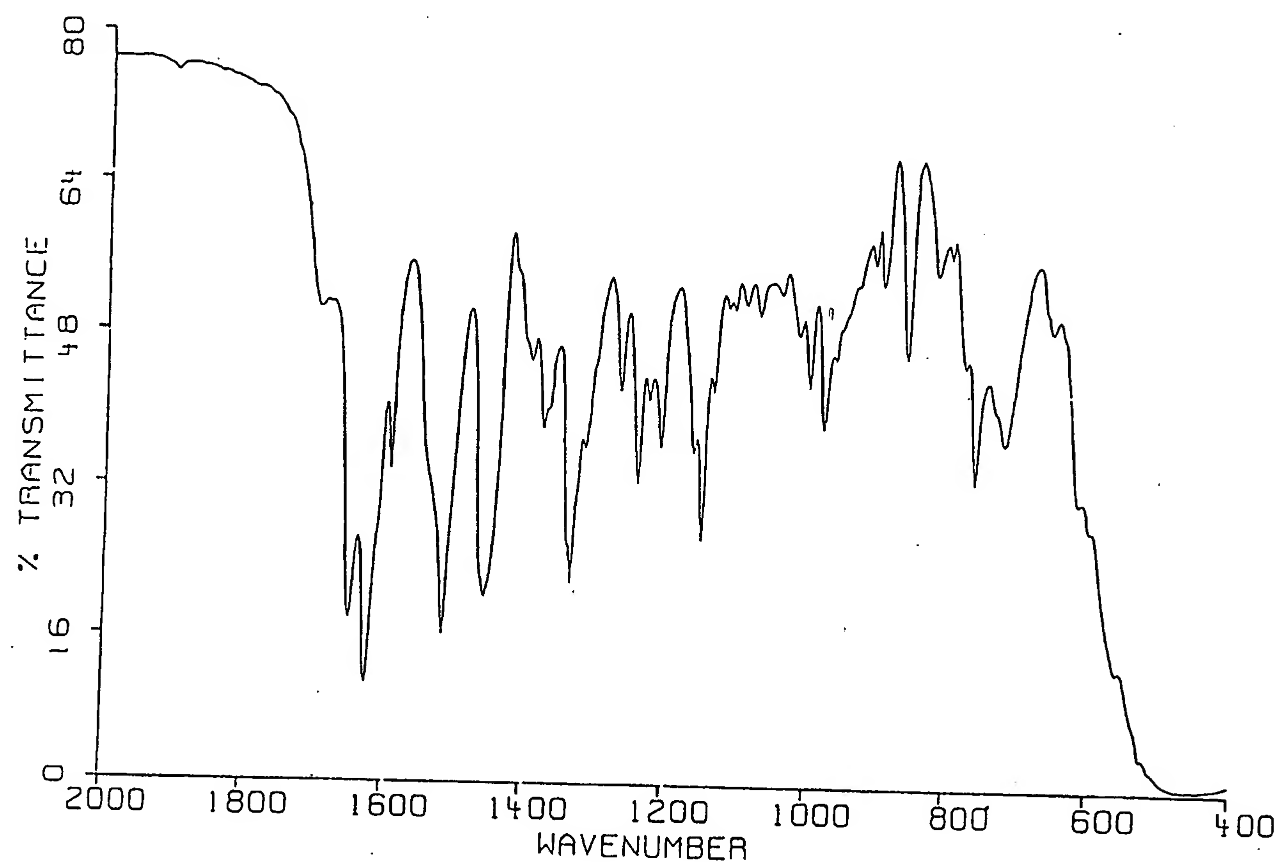


FIGURE 2A

IR spectrum of the β-form as a nujol mull on a sodium chloride disc with air as the reference
(ν = 4000-400cm^{-1})

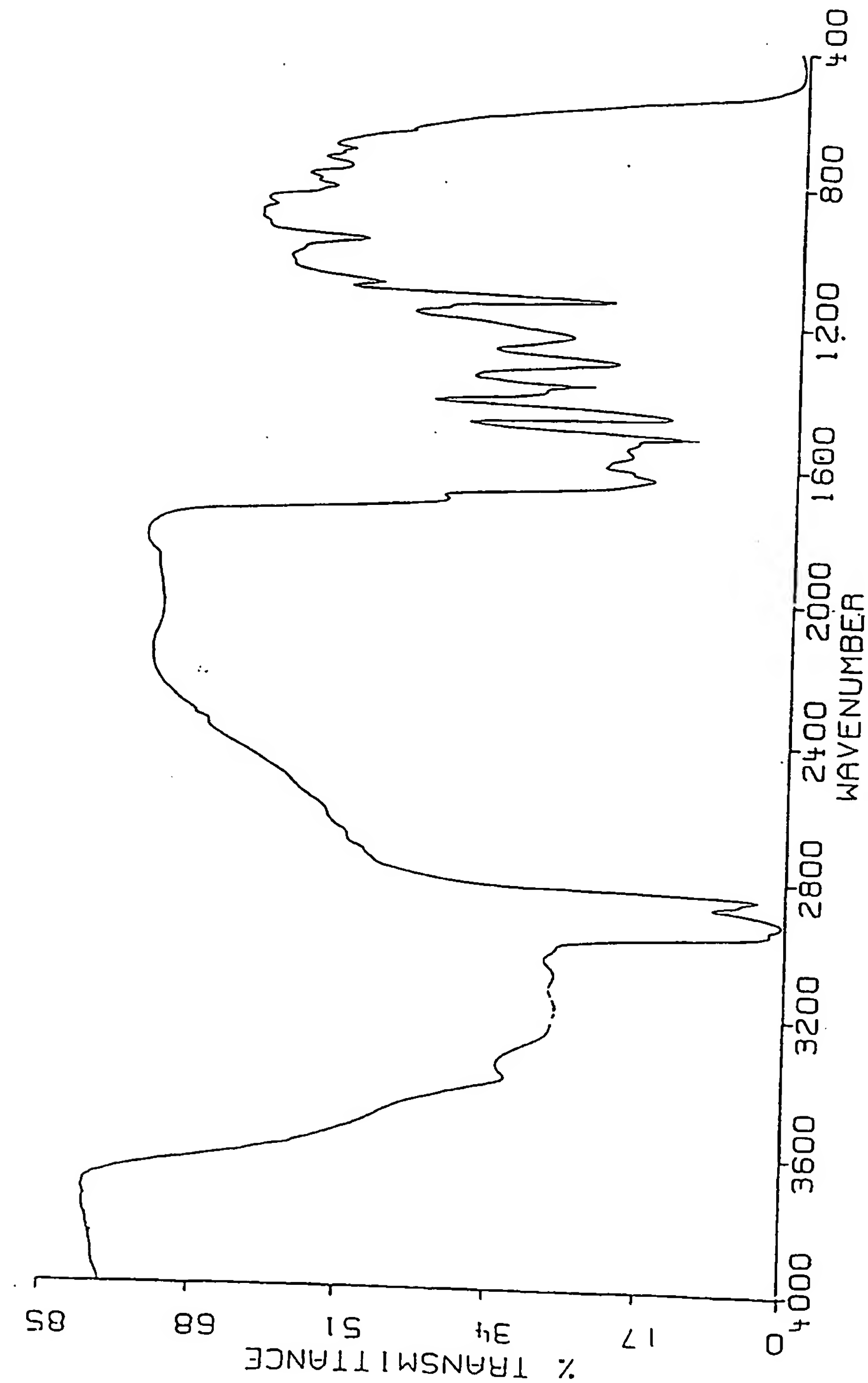


FIGURE 2B

IR spectrum of the ß-form as a nujol mull on a sodium chloride disc with air as the reference
(ν = 2000-400cm^{-1})

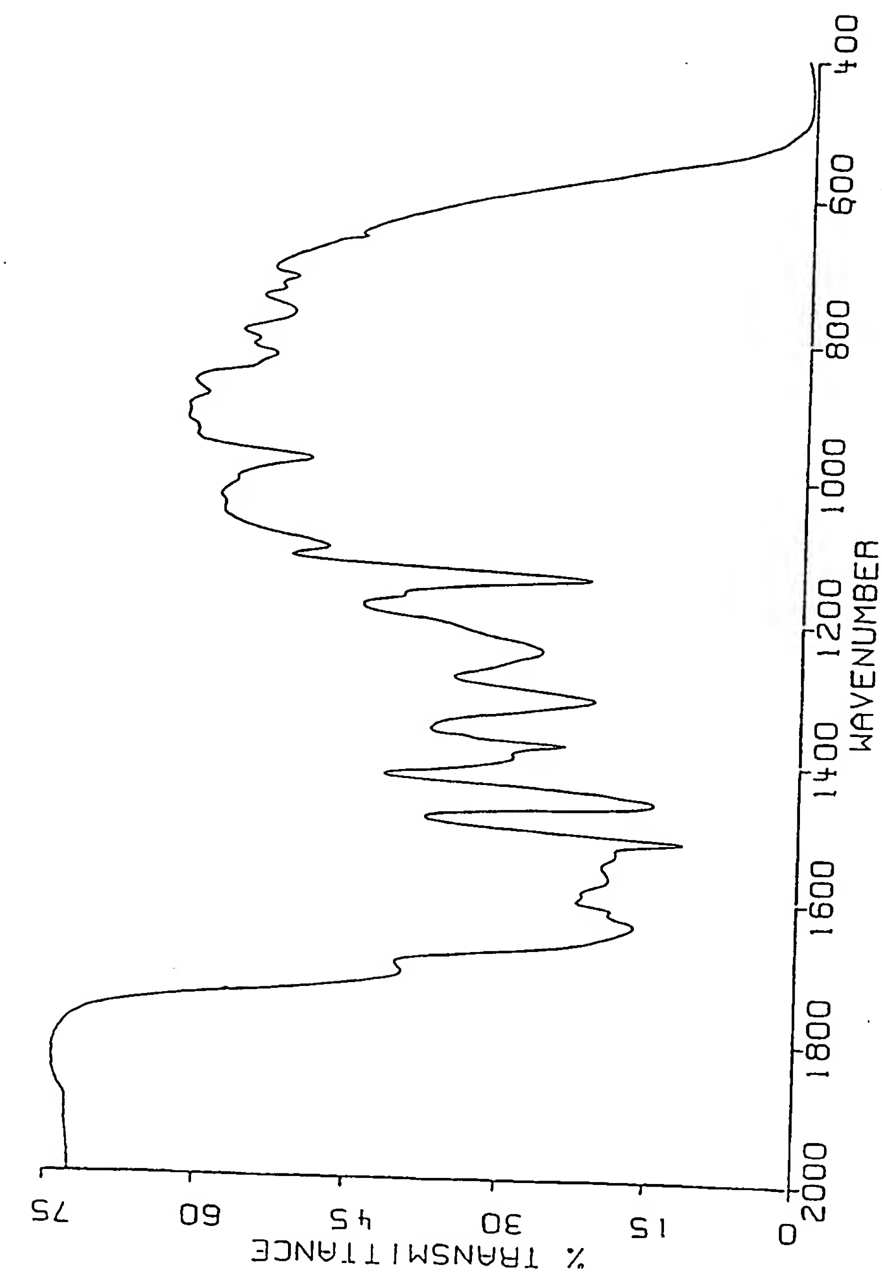


FIGURE 3A

IR spectrum of the γ-form as a nujol mull on a sodium chloride disc with air as the reference (ν = 4000-400cm^{-1})

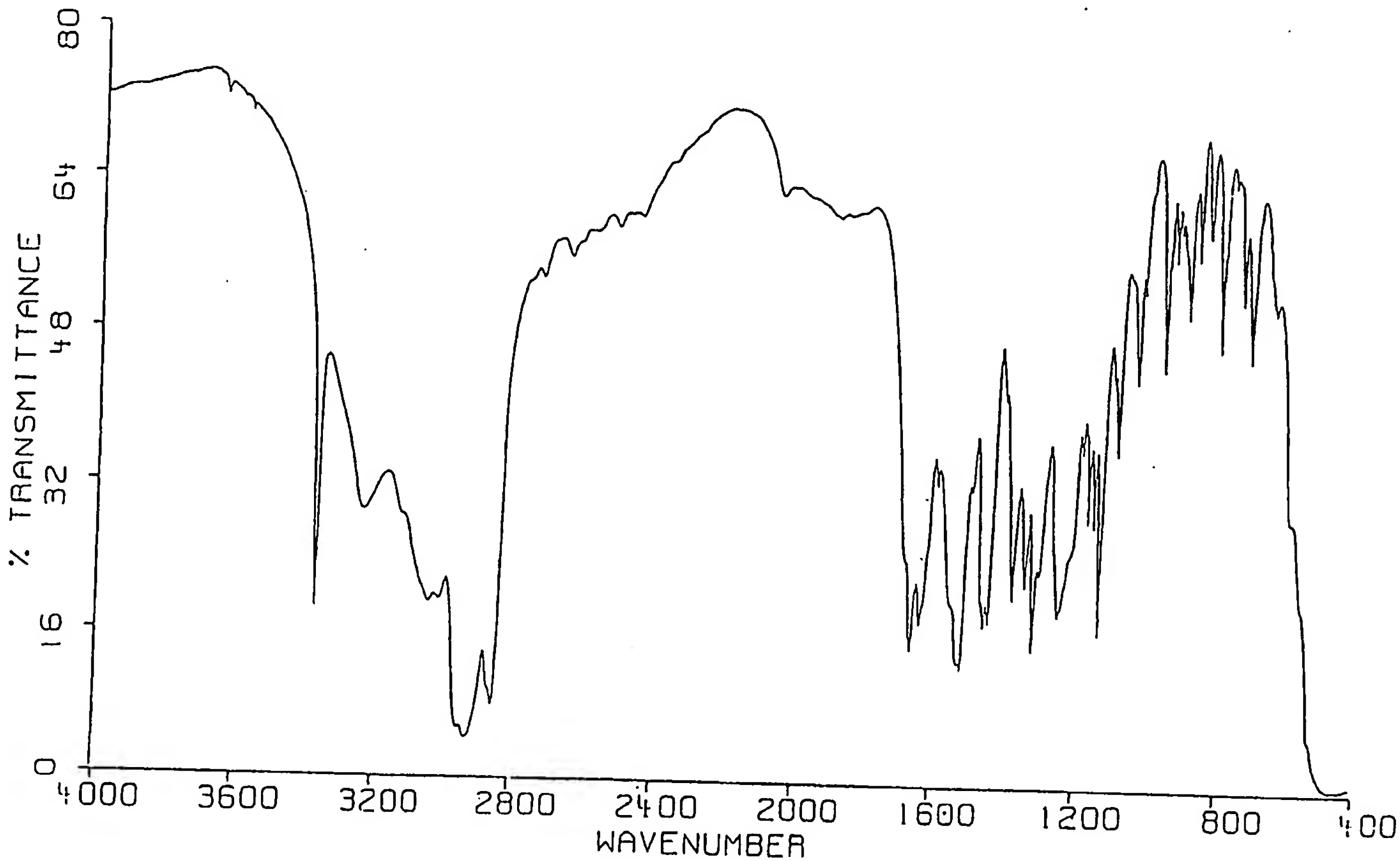


FIGURE 3B

IR spectrum of the γ-form as a nujol mull on a sodium chloride disc with air as the reference
(ν = 2000-400cm^{-1})

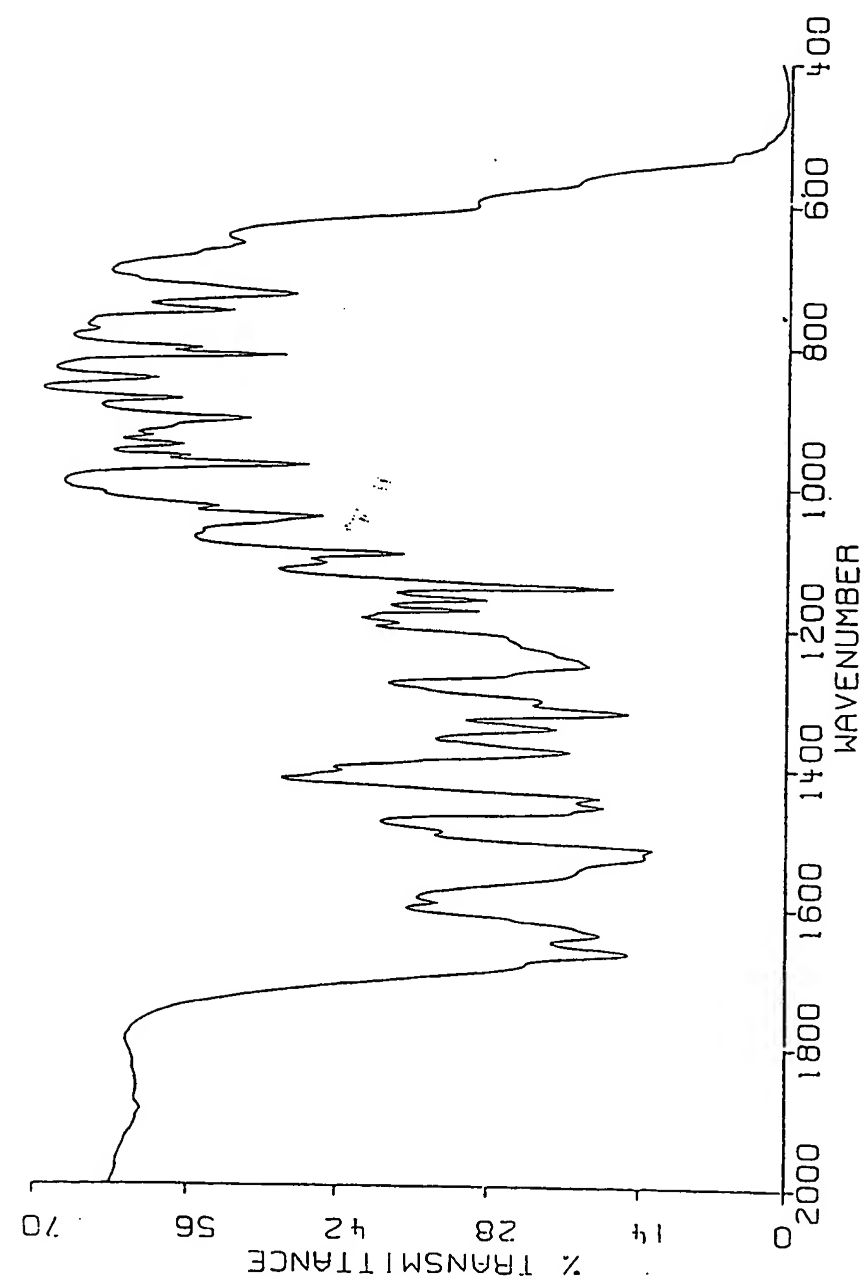


FIGURE 4A

IR spectrum of the δ-form as a nujol mull on a sodium chloride disc with nujol on sodium chloride disc reference (ν = 4000-400cm^{-1})

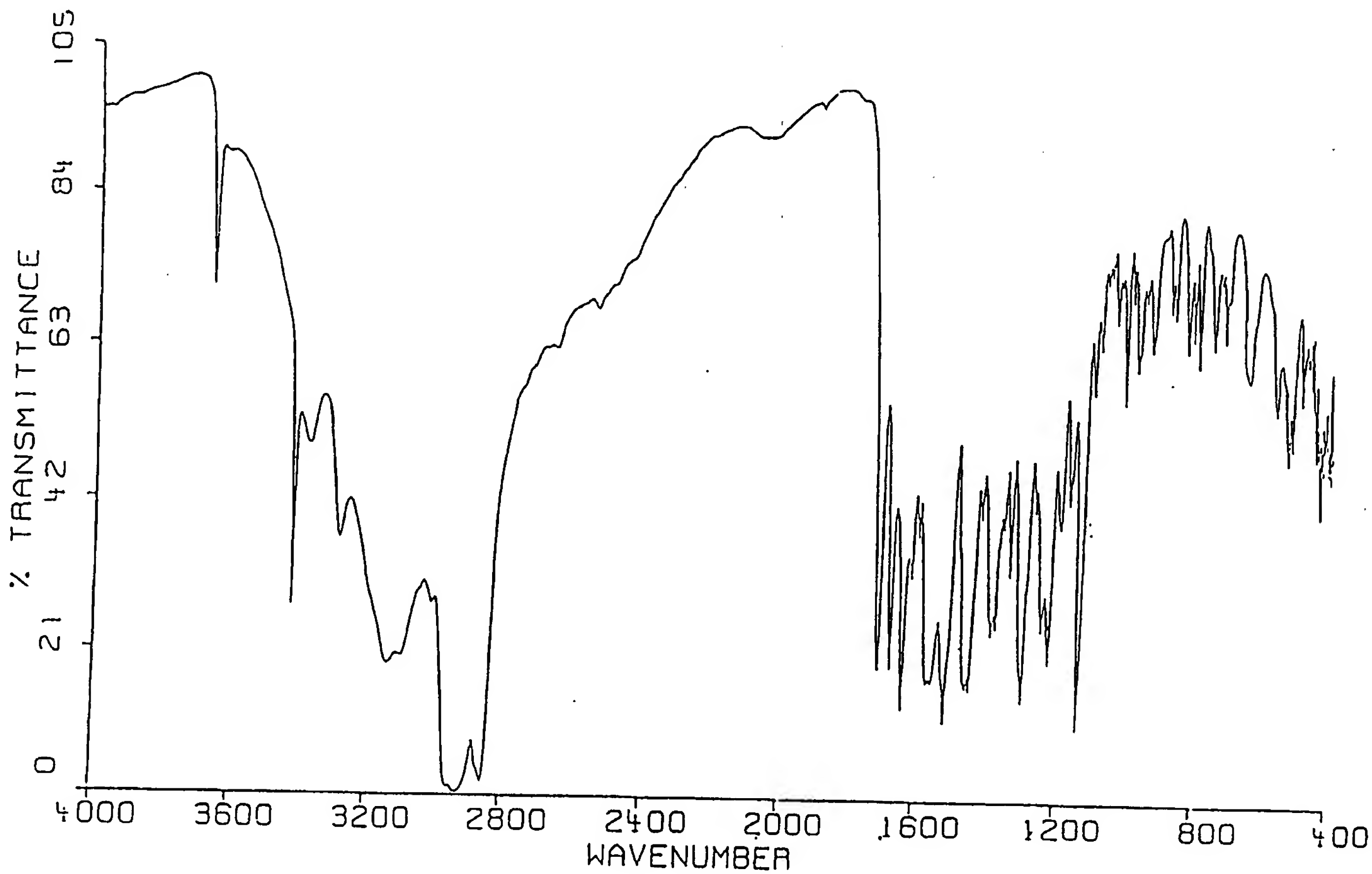


FIGURE 4B

IR spectrum of the δ-form as a nujol mull on a sodium chloride disc with a nujol on sodium chloride disc reference (ν = 2000–400cm^{-1})

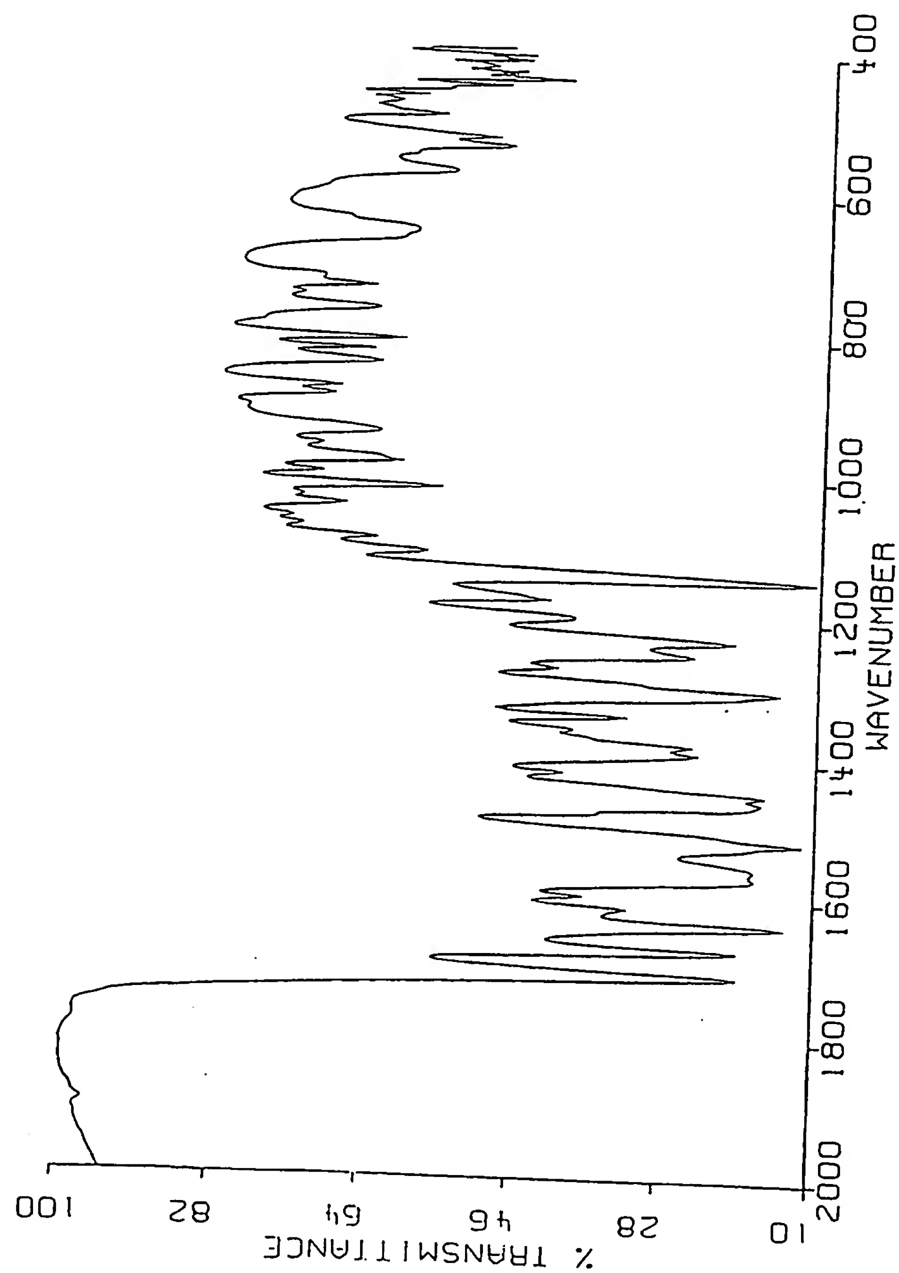


FIGURE 5

PXRD of the α-form

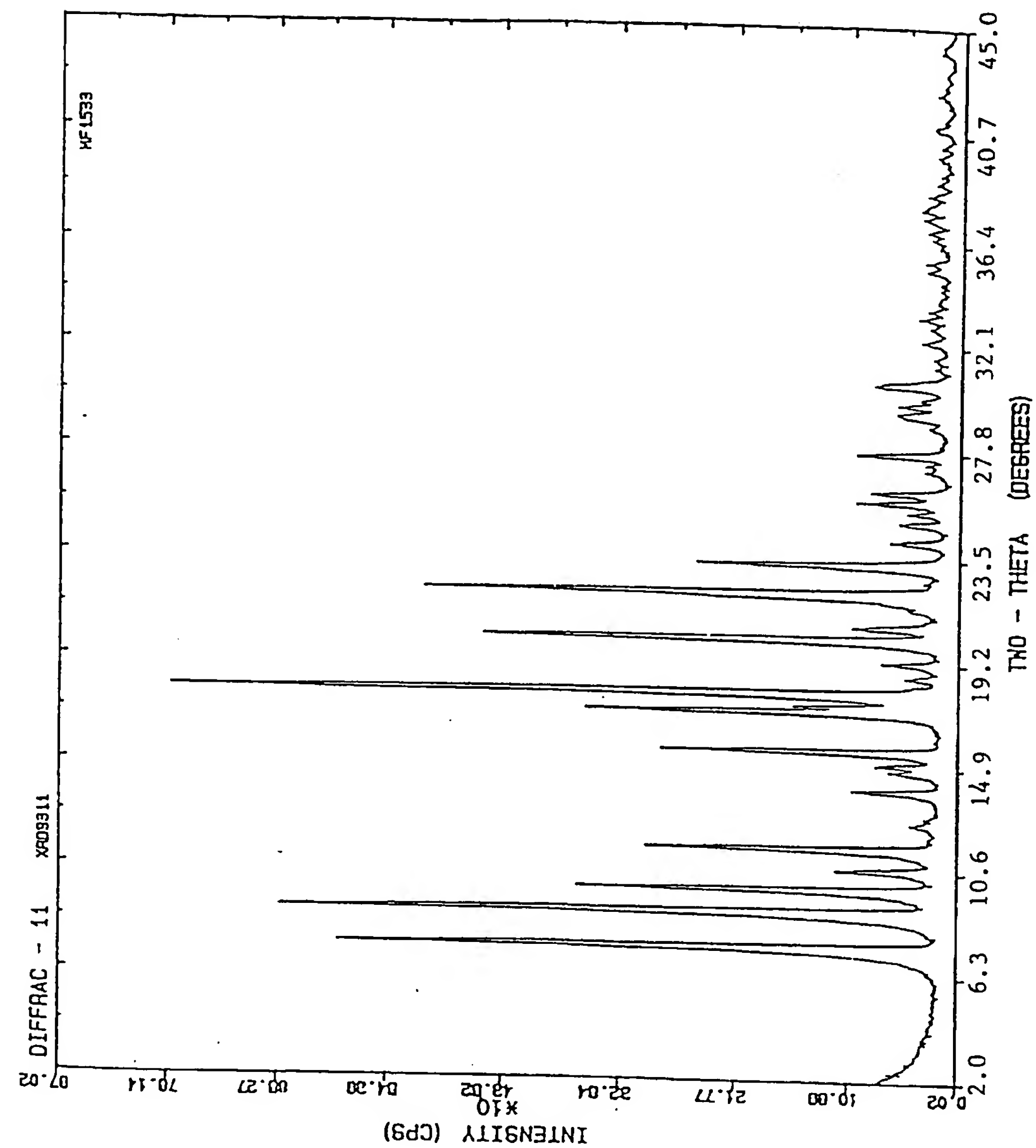


FIGURE 6

PXRD of the ß-form

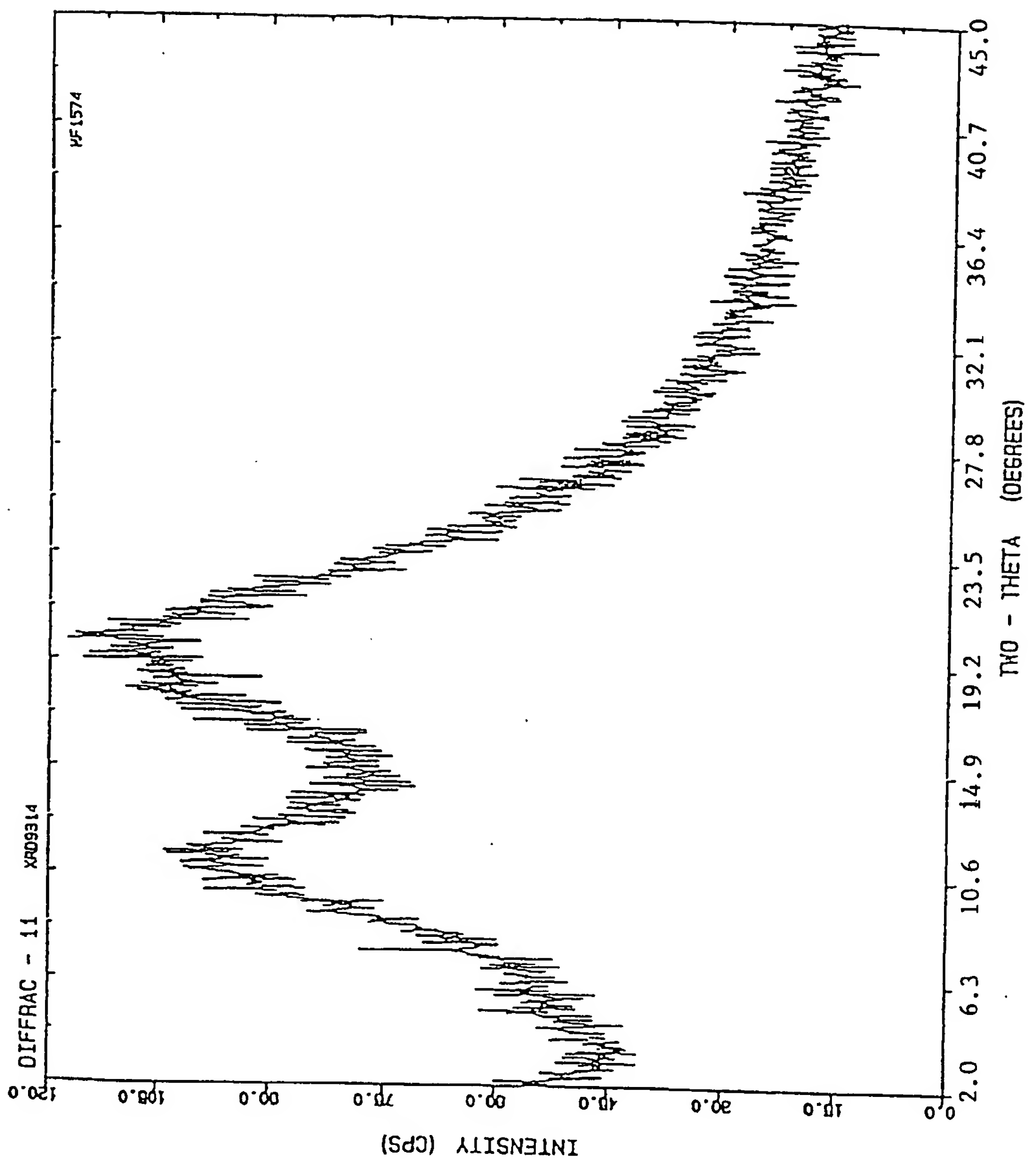


FIGURE 7

PXRD of the γ-form

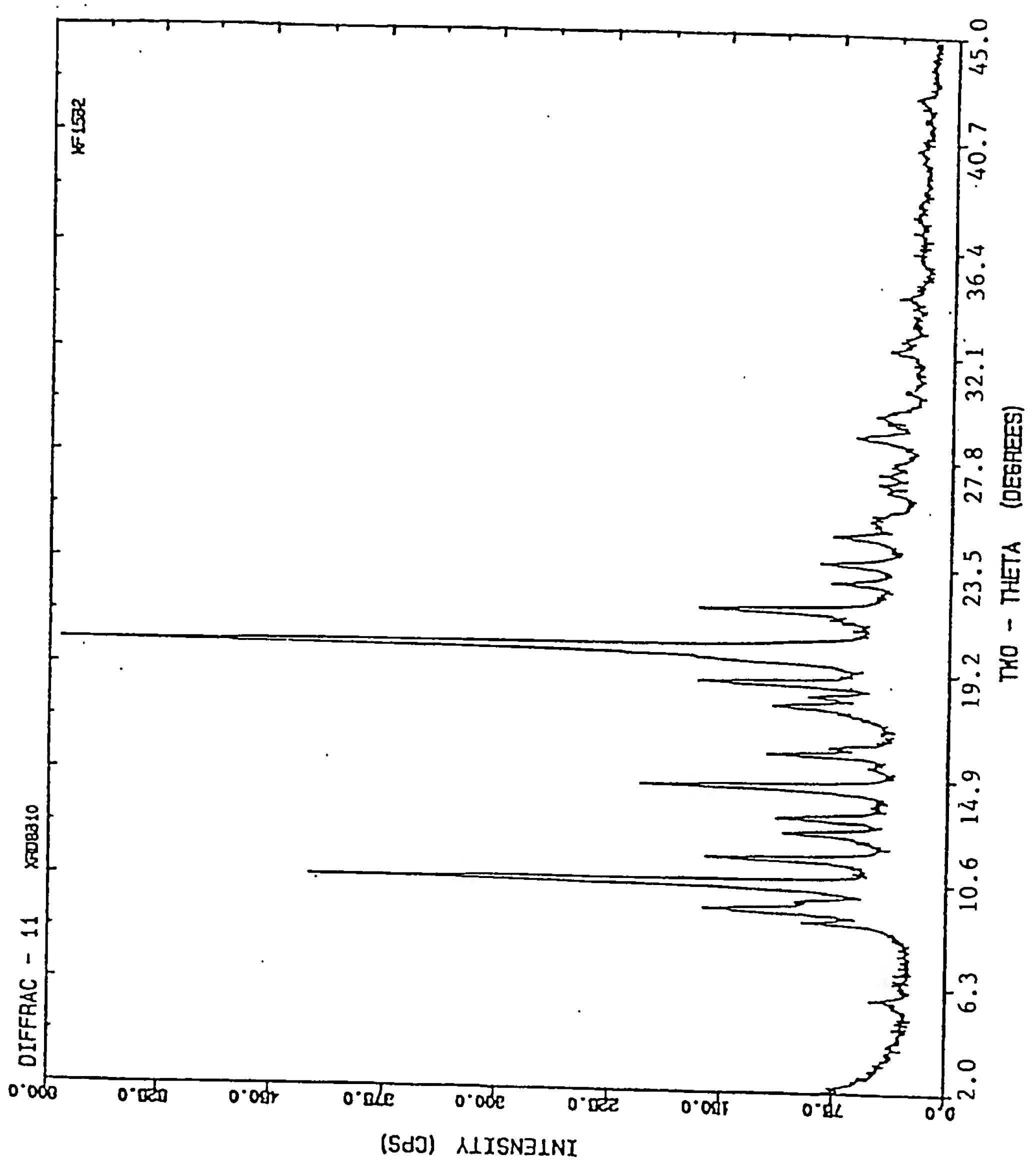


FIGURE 8

PXRD of the δ-form

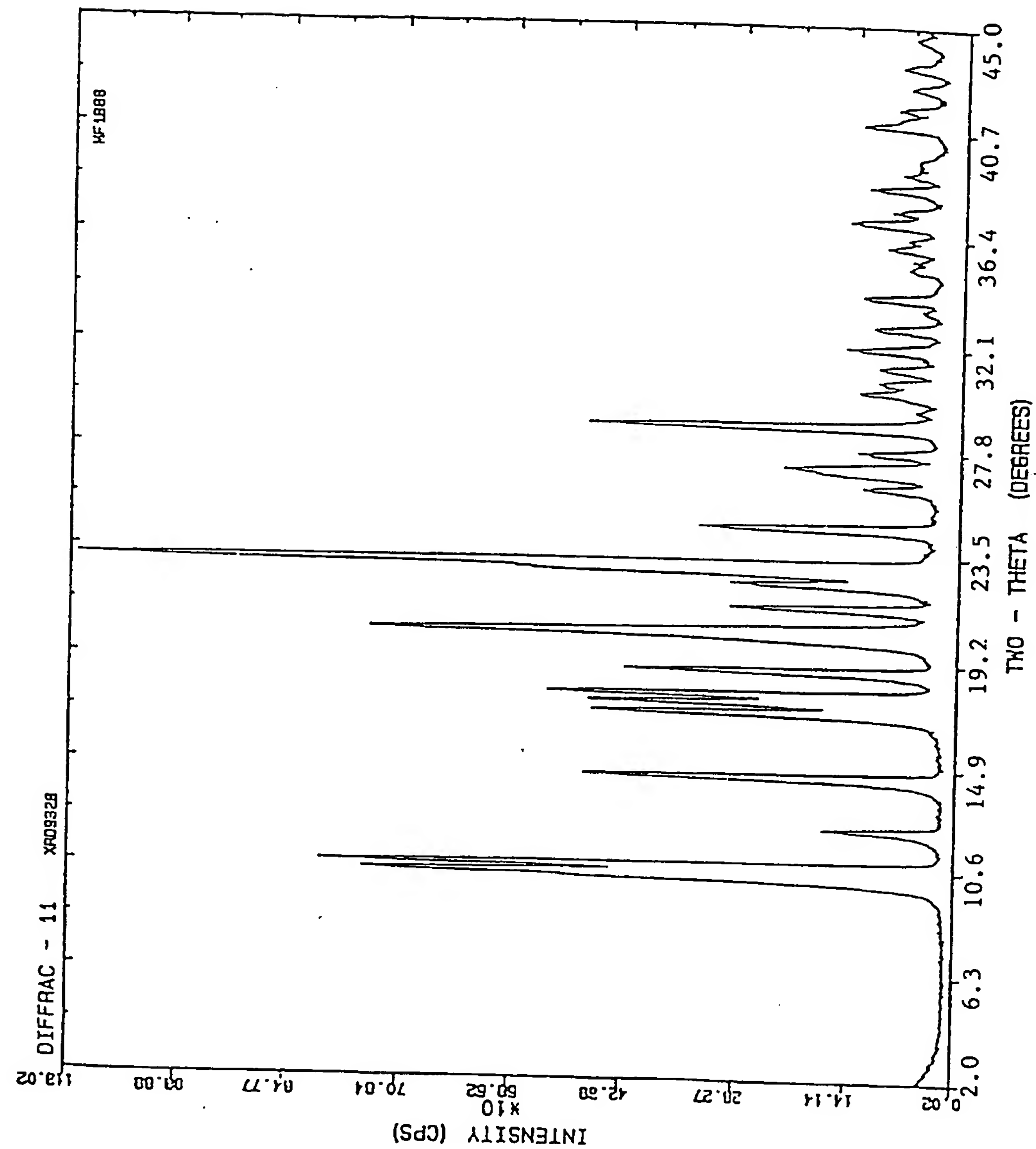


FIGURE 9

DSC thermogram of the α-form

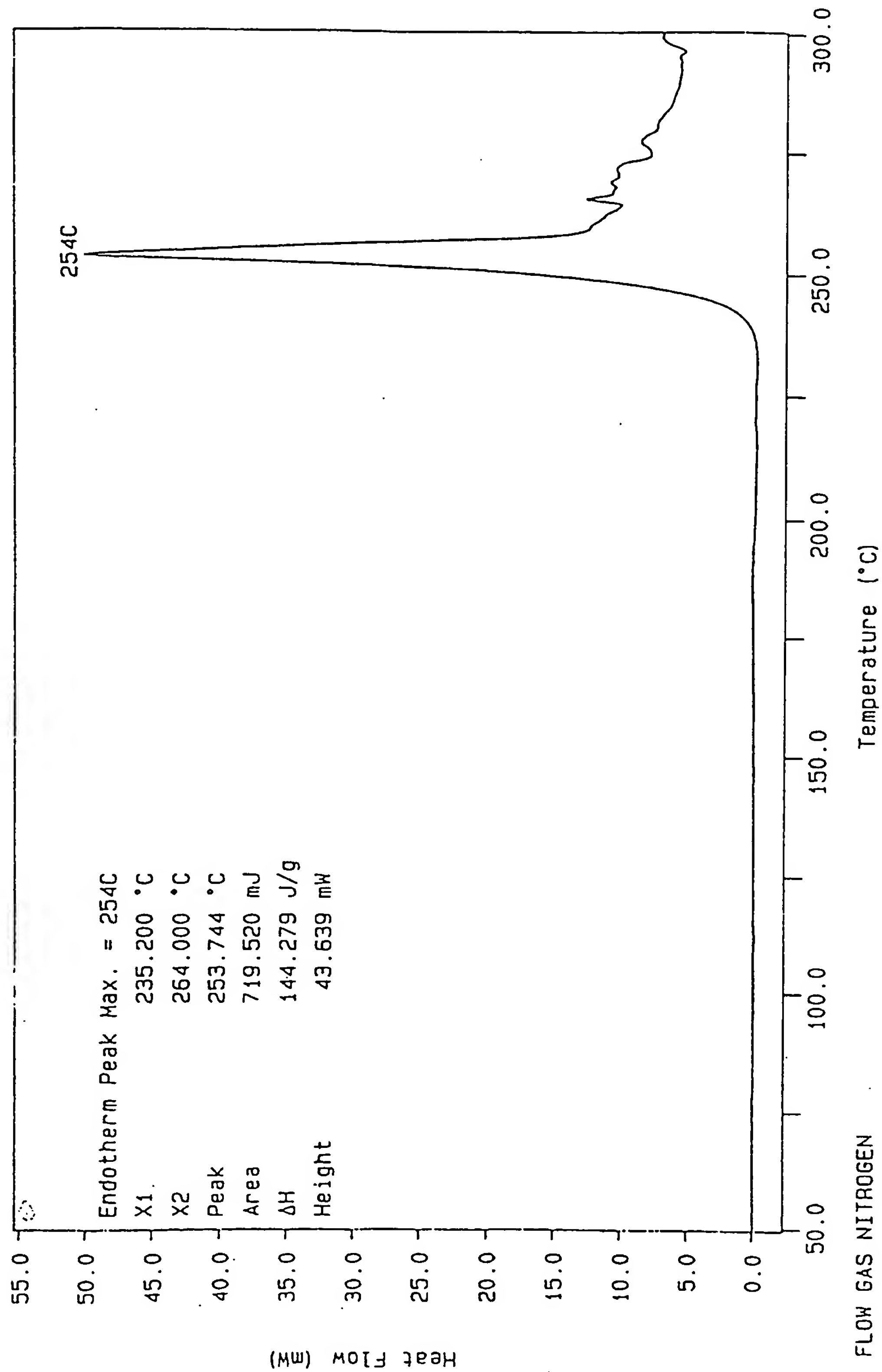


FIGURE 10

DSC thermogram of ß-form

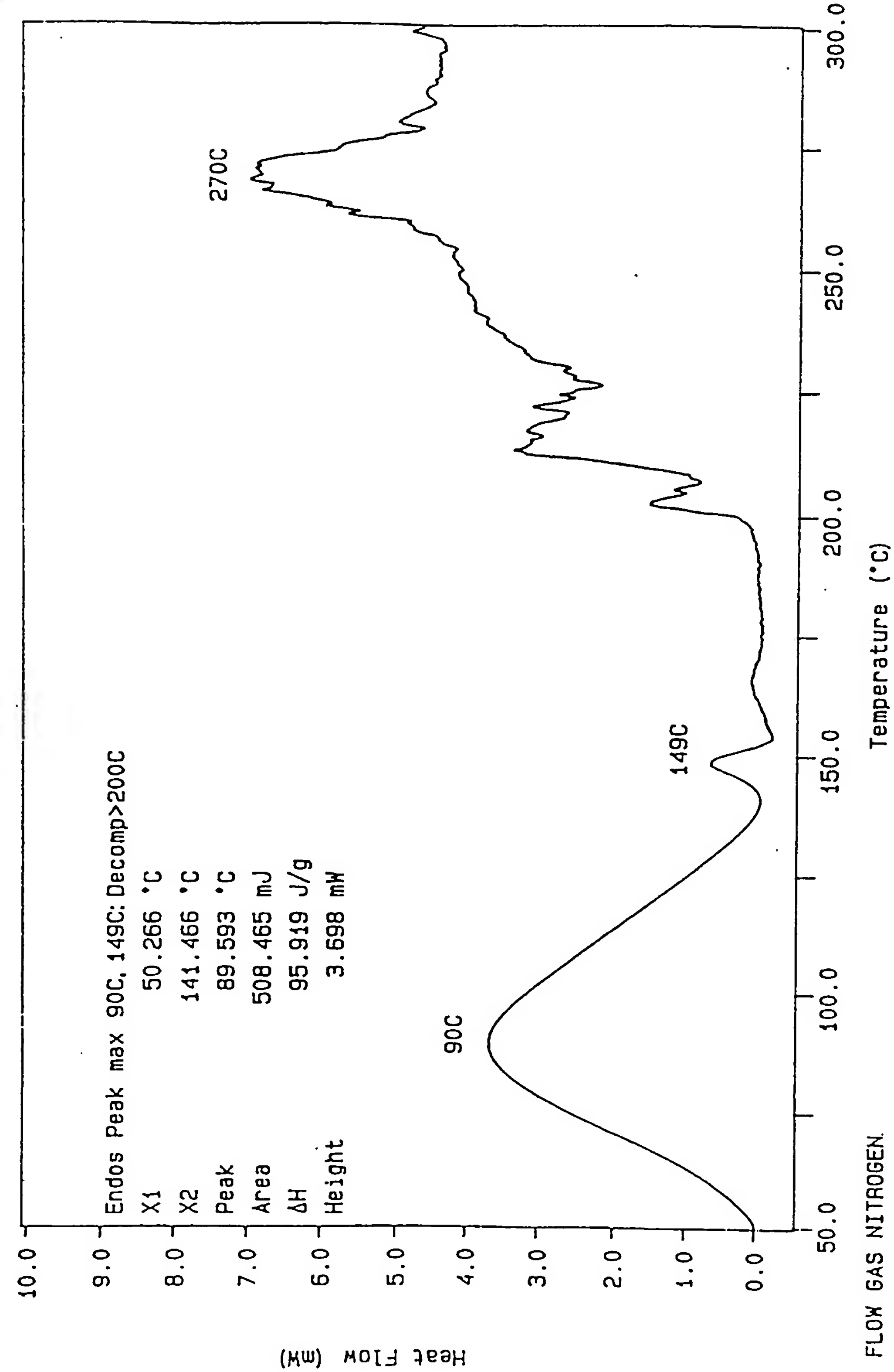


FIGURE 11

DSC thermogram of γ-form

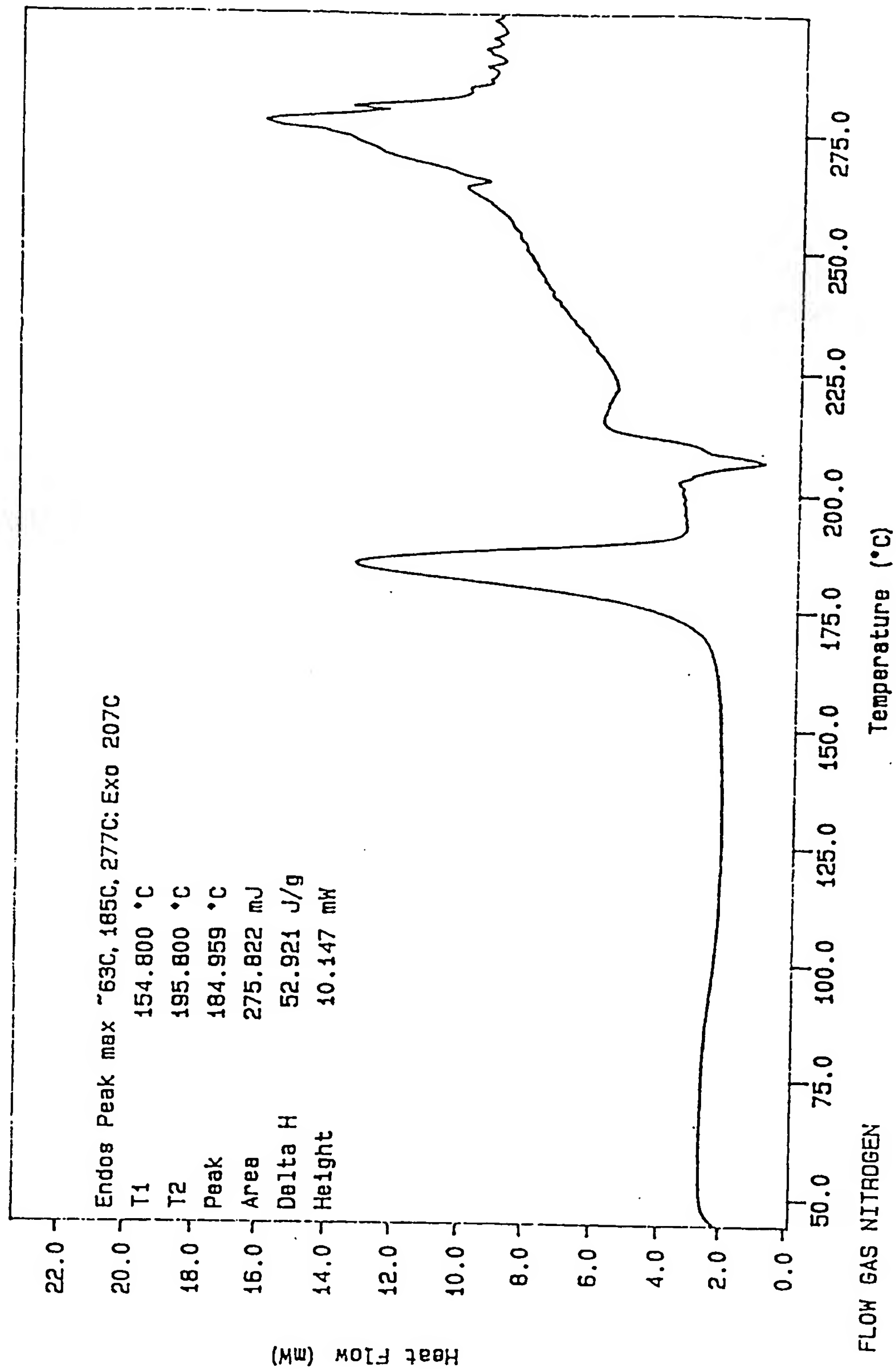


FIGURE 12

DSC thermogram of δ-form

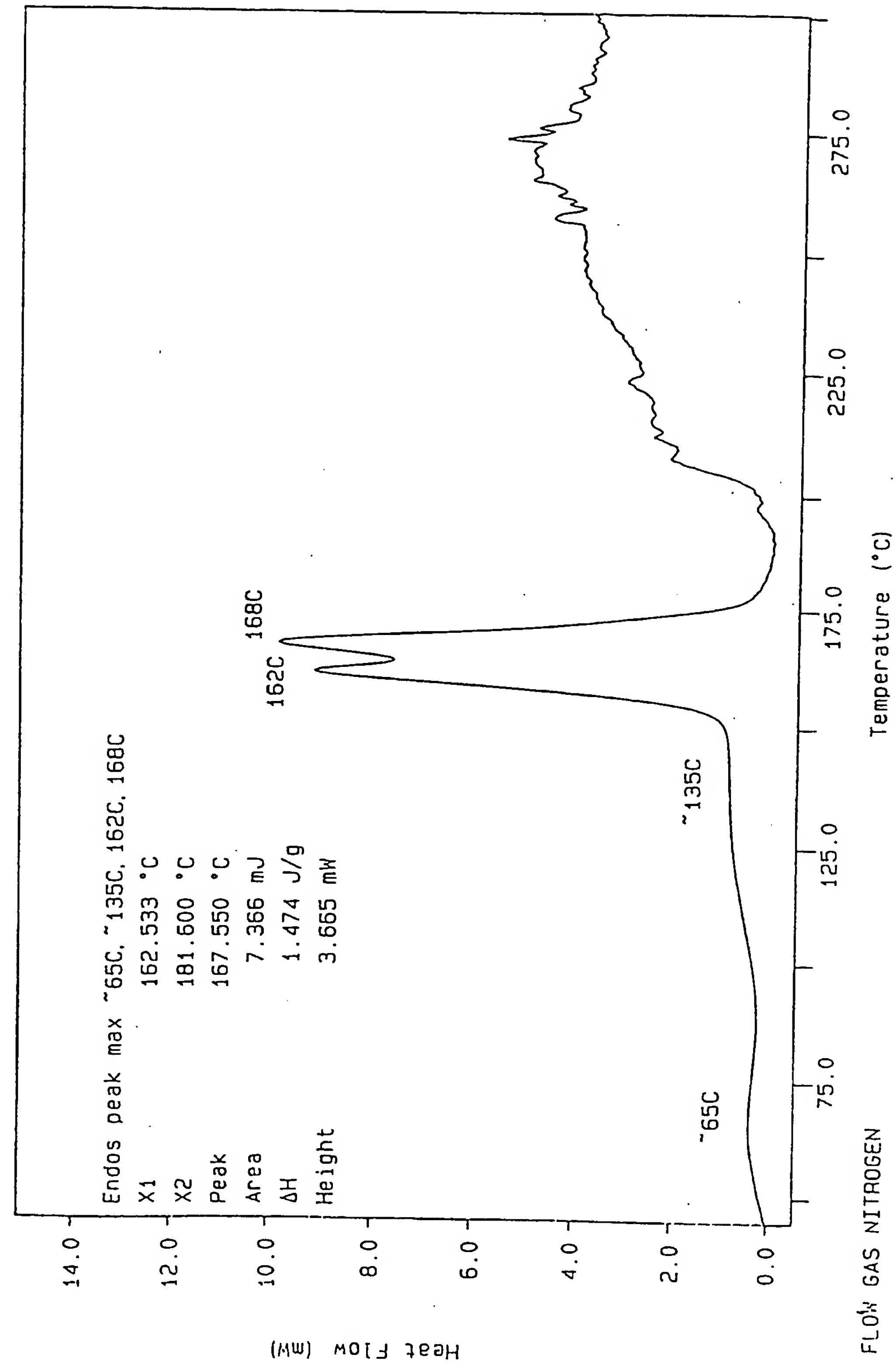


FIGURE 13

Graph plotting upper punch force as a function of number of tablets for an Avicel/DCP placebo blend

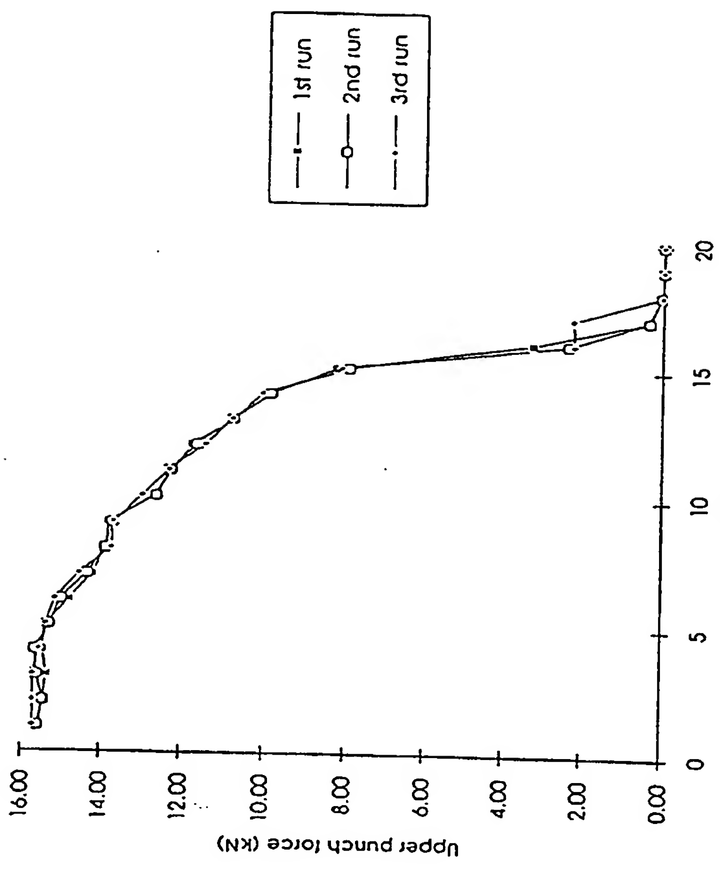


FIGURE 14

Graph plotting upper punch force as a function of number of tablets for α- and β-form blends

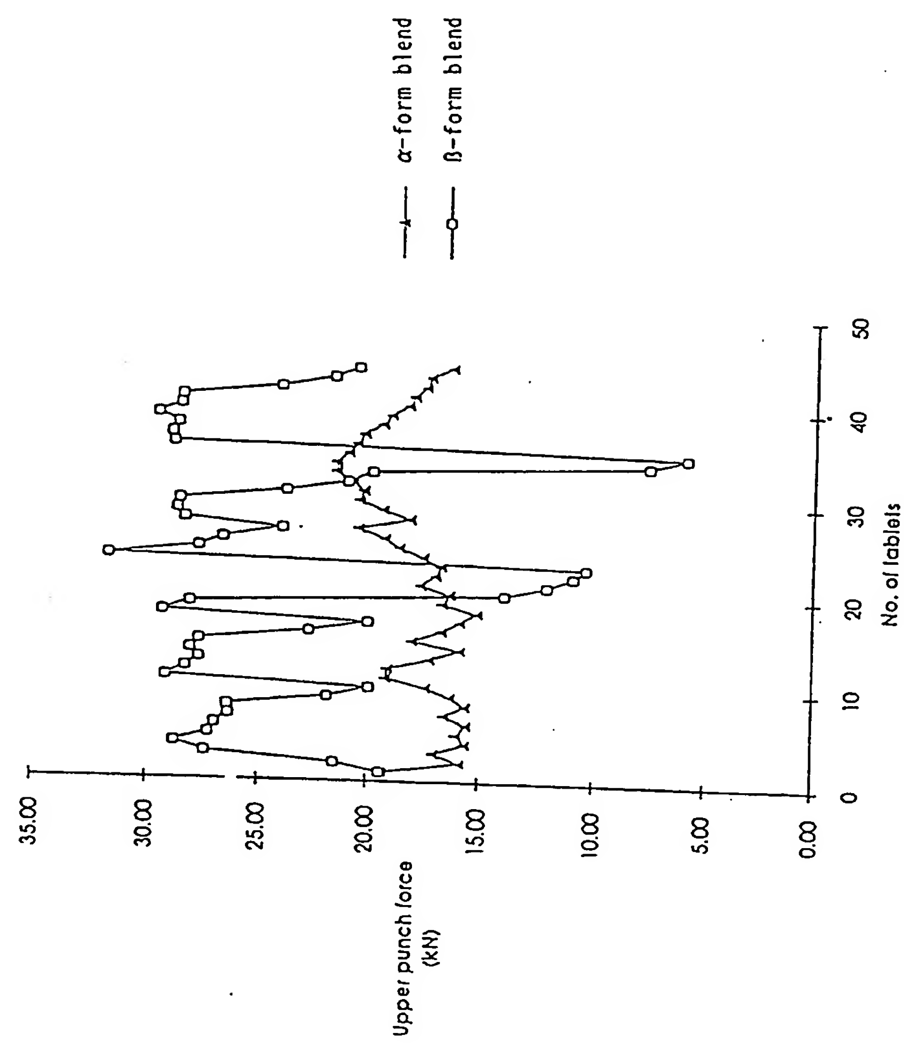


FIGURE 15

Graph plotting ejection force (kN) as a function of number of tablets for the α- and β-form blends

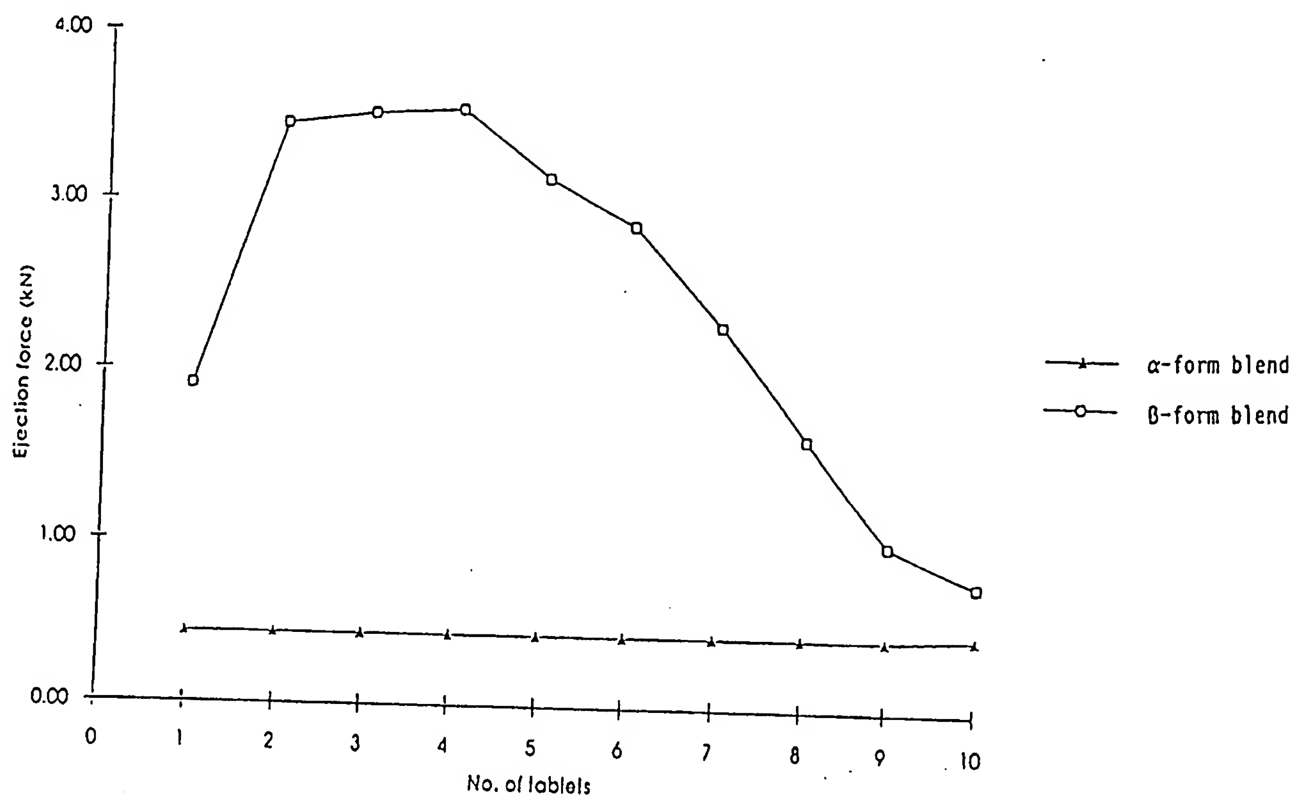


FIGURE 16

Graph plotting mean percentage change in weight as a function of time for the α- and β-forms at 40°C and at various relative humidities (RH)

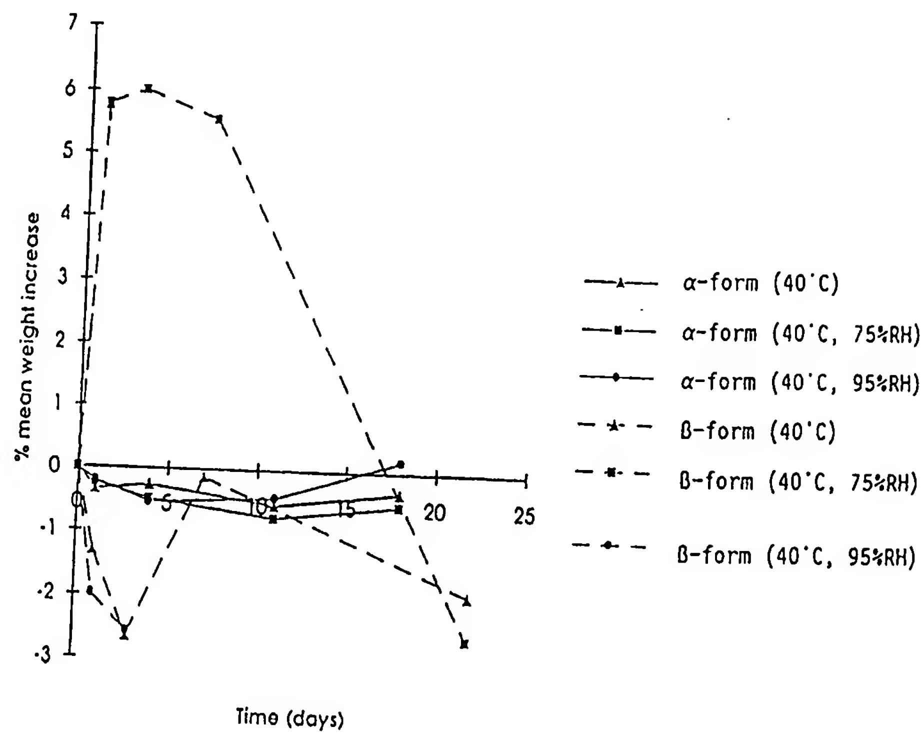
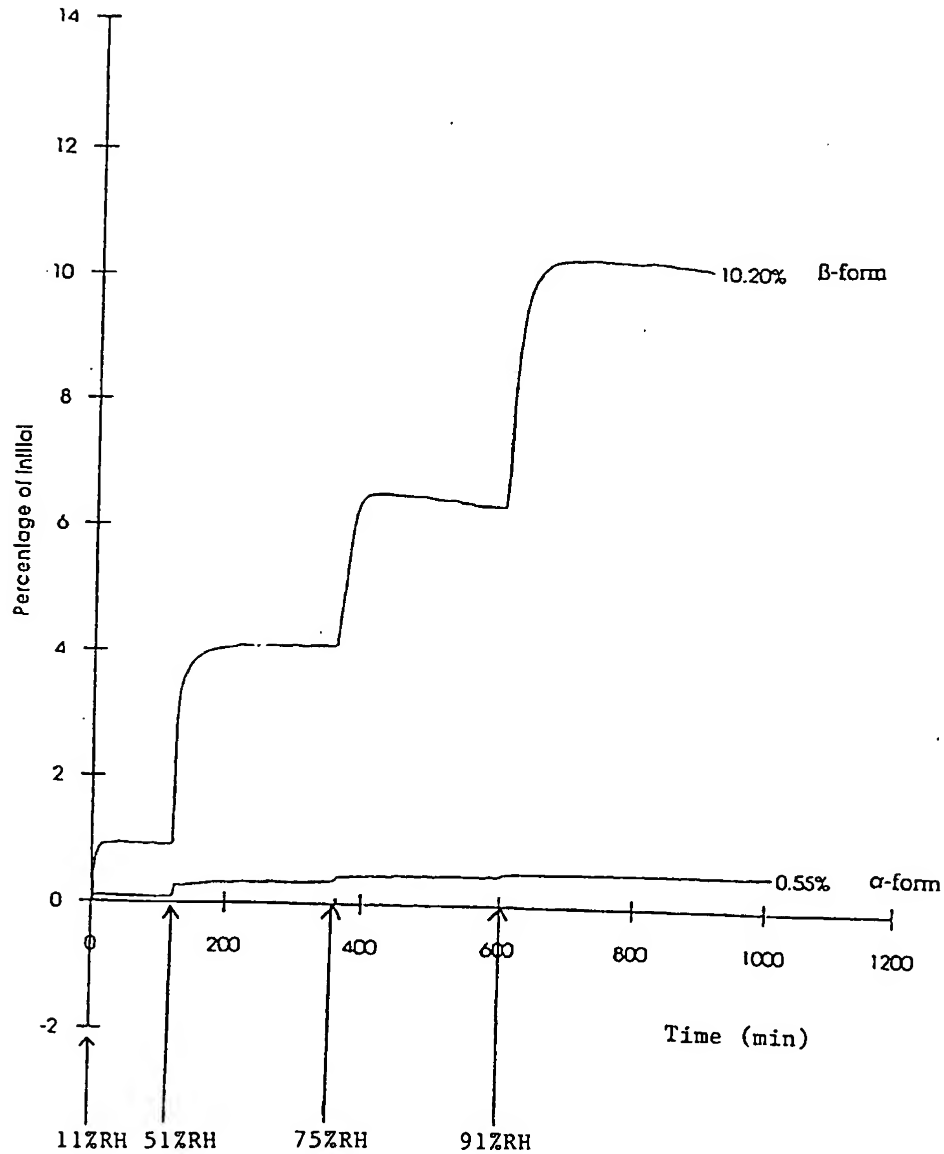


FIGURE 17

Moisture sorption of the α- and ß-forms at 40°C with exposure to increasing relative humidities



INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 94/03750

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07C311/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 358 398 (PFIZER LTD) 14 March 1990 cited in the application see example 181 see examples 103,108-114	1-7
P,A	WO,A,94 06756 (SCHERING CORP.) 31 March 1994 see claims 1-4	17-23

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
25 January 1995	22.02.95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Goetz, G

INTERNATIONAL SEARCH REPORT
Information on patent family members

Inte   onal Application No
PCT/EP 94/03750

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0358398	14-03-90	AU-B-	604195	06-12-90
		AU-A-	4105289	08-03-90
		ES-T-	2054009	01-08-94
		JP-A-	2124862	14-05-90
		JP-B-	6060144	10-08-94
WO-A-9406756	31-03-94	US-A-	5208236	04-05-93
		AU-B-	5128793	12-04-94